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“EVOLUCIÓN DE LA FISIOLOGÍA TÉRMICA E HISTORIAS DE VIDA EN LAS LAGARTIJAS DE LA FAMILIA PHRYNOSOMATIDAE”

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

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Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **19 de abril de 2020** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **DOMÍNGUEZ GUERRERO SAÚL FILEMÓN** con número de cuenta **516012306** con la tesis titulada **"EVOLUCIÓN DE LA FISIOLOGÍA TÉRMICA E HISTORIAS DE VIDA EN LAS LAGARTIJAS DE LA FAMILIA PHRYNOSOMATIDAE",** realizada bajo la dirección del **DR. FAUSTO ROBERTO MÉNDEZ DE LA CRÚZ,** quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
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Hembra adulta del Camaleón de Montaña, *Phrynosoma orbiculare*, fotografiada en la Localidad de El Encinal, Cuautepec de Hinojosa, Hidalgo, México.

“*Nothing in biology makes sense except in the light of evolution*”.
Theodosius Dobzhansky (1900-1975).

ÍNDICE

RESUMEN.

Los animales ectotermos dependen de la temperatura ambiental para regular su temperatura corporal (T_b), por ello son susceptibles al cambio climático (CC). Es posible que los organismos ectotermos respondan al CC mediante ajustes conductuales y/o adaptación de su fisiología térmica. En la presente tesis se puso a prueba la efectividad en la termorregulación conductual y la adaptación de la temperatura corporal preferida (T_{pref}), la tolerancia al frío (CT_{min}) y la tolerancia al calor (CT_{max}) en lagartijas de la familia Phrynosomatidae. Se documentó que mediante termorregulación conductual los individuos evaden temperaturas letales y mantienen su T_b dentro de su intervalo de T_{pref}. Dicho intervalo de T_{pref} evoluciona como respuesta a la temperatura ambiental en especies ovíparas, pero no en especies vivíparas. A mayor temperatura ambiental, las especies ovíparas prefieren temperaturas más altas, pero independientemente del ambiente térmico, las especies vivíparas prefieren temperaturas bajas. Para explorar por qué la T_{pref} evoluciona de forma distinta entre modos de paridad se integró información metabólica y de historias de vida de las especies bajo estudio. Mediante modelos evolutivos se documentó que las especies vivíparas tienen una tasa metabólica menor que las especies ovíparas, resultado de sus preferencias por temperaturas corporales bajas, lo que podría ser una compensación energética a una gestación intrauterina completa. Además, la tasa metabólica baja de las especies vivíparas estuvo asociada con una menor fecundidad anual. Con respecto a la evolución de las tolerancias térmicas, se encontró que la CT_{min} es una adaptación a la temperatura ambiental, pero la CT_{max} no varía en función del ambiente térmico. Ya que la CT_{max} no cambia de forma adaptativa a la temperatura ambiental y que la T_{pref} no evoluciona más allá de la CT_{max}, los phrynosomatidos son susceptibles al calentamiento global (tendencia del CC), especialmente las especies vivíparas que deben mantener temperaturas corporales bajas debido a sus requerimientos metabólicos y reproductivos.

Palabras clave: Adaptación, Cambio climático, Temperatura corporal preferida, Tolerancia al calor, Tolerancia al frío, Viviparidad.

ABSTRACT

Ectothermic animals depend of environmental temperature to regulate their body temperature (T_b), and because of this, they are susceptible to climate change (CC). It is possible that ectothermic organisms respond to CC through behavioral adjustments and/or adaptation in thermal physiology. In this thesis, we tested effectiveness in behavioral thermoregulation and adaptation in preferred body temperature (T_{pref}), cold tolerance (CT_{min}) and heat tolerance (CT_{max}) of Phrynosomatidae lizards. We found that individuals avoid lethal temperatures and maintain their T_b within their T_{pref} range through behavioral thermoregulation. T_{pref} range evolves in response to environmental temperature in oviparous species, but does not in viviparous species. To higher environmental temperature, oviparous species prefer higher body temperatures, but independently of thermal environment, viviparous species always prefer lower body temperatures. To explore why T_{pref} evolves in a distinct way between parity modes, we include metabolic and life history data. Through evolutionary models, we found that viviparous species exhibit a lower metabolic rate than oviparous species, related to lower body temperatures, which could be an energetic compensation to a complete intrauterine gestation. Furthermore, a lower metabolic rate in viviparous species was associated with a lower annual fecundity. With respect to evolution of thermal tolerances, we found that CT_{min} is an adaptation to environmental temperature, but CT_{max} is not variable in response to thermal environment. Because CT_{max} is not an adaptation to environmental temperature and that T_{pref} does not evolve beyond CT_{max}, phrynosomatids are susceptible to global warming (tendency of CC), in special viviparous species, which need to maintain lower body temperatures, related to their metabolic and reproductive requirements.

Key words: Adaptation, Cold tolerance, Climate change, Preferred body temperature, Heat tolerance, Viviparity.

INTRODUCCIÓN GENERAL.

El cambio climático (CC)—“cambio de clima atribuido directa o indirectamente a la actividad humana que altera la composición de la atmósfera global y que se suma a la variabilidad natural del clima observada durante periodos de tiempo comparables”—está ocasionando perdida de la biodiversidad a nivel mundial[1]. Los animales ectotermos son especialmente susceptibles al CC y en algunos grupos se ha proyectado que al menos el 20% de las especies conocidas se habrán extinto en el presente siglo, a menos que las poblaciones presenten respuestas adaptativas[2,3]. La tasa de extinción proyectada para animales ectotermos es alarmante, considerando que representan más del 95% de la diversidad animal en el mundo[4]. Los organismos ectotermos son susceptibles al cambio climático debido a que dependen de la temperatura ambiental para regular su temperatura corporal (T_b)[5,6,7]. Si los individuos mantienen su T_b dentro de su intervalo de temperatura corporal preferida (T_{pref}), entonces su supervivencia y éxito reproductor se maximizan[8]. Sin embargo, la adecuación de los individuos decrece (hasta volverse nula) a temperaturas corporales bajas (temperatura crítica mínima; CT_{min}) o altas (temperatura crítica máxima; CT_{max})[5,9]. Así, los cambios en el ambiente térmico pueden ocasionar que los animales ectotermos excedan sus límites de tolerancia térmica (CT_{min} y CT_{max}) y mueran de frío o calor, o que los individuos limiten su horario de actividad (evitando temperaturas letales), pero al no tener tiempo suficiente para alimentarse, no ganen energía suficiente para reproducirse, no exista reclutamiento poblacional y las poblaciones decrezcan hasta su extinción[10,11]. A pesar del escenario poco alentador por el cambio climático y extinción de especies, se ha propuesto que las poblaciones de animales ectotermos podrían responder mediante adaptación[6,12,13].

Dos respuestas adaptativas podrían permitir la subsistencia de los animales ectotermos al cambio climático: ajustes conductuales y plasticidad de la fisiología térmica[3,6]. Mediante ajustes conductuales, por ejemplo, cambio en el uso de sustrato para termorregular o cambio en el horario de actividad, los animales ectotermos podrían mantener su T_b dentro de su intervalo de T_{pref}, evadir temperaturas letales, sobrevivir y reproducirse, incluso en ambientes térmicos extremos[14,15,16]. Mediante plasticidad de la fisiología térmica, por ejemplo, cambio de las preferencias térmicas de los individuos, correlacionado positivamente con la temperatura ambiental, los animales ectotermos podrían maximizar su desempeño y adecuación en ambientes térmicos variables[13,17,18,19]. Ambas respuestas adaptativas (conductuales y fisiológicas) pueden ser puestas a prueba con estudios poblacionales e interespecíficos. Por una parte, los estudios poblaciones permiten evaluar si los individuos mantienen con efectividad su T_b dentro de su intervalo de T_{pref}, y al mismo tiempo evaluar si a través su periodo de vida su T_{pref}, CT_{min} y/o CT_{max} cambian de forma correlacionada (positivamente) con la temperatura ambiental. Por otra parte, los estudios interespecíficos permiten poner a prueba si la selección natural favorece ajustes conductuales y fisiológicos (en CT_{min}, T_{pref} y CT_{max}) similares en especies lejanamente emparentadas, pero que habitan ambientes térmicos parecidos (*i.e.* convergencias evolutivas). Ambos métodos (poblacional e interespecífico) son complementarios y ayudarán a entender de mejor manera si los ajustes conductuales y la plasticidad fisiológica de los ectotermos les permitirán responder al cambio climático.

En la presente tesis, se eligió como modelo de estudio a las especies de lagartijas de la familia Phrynosomatidae. La familia Phrynosomatidae está integrada por 163 especies distribuidas de norte a centro América y elevaciones que varían desde el nivel del mar hasta cerca de los 5000 metros[20,21,22]. Se esperaría que, si a nivel poblacional los phrynosomatidos presentan ajustes conductuales y plasticidad fisiológica como respuesta a la temperatura ambiental, entonces esos ajustes conductuales y fisiológicos se verían reflejados en patrones adaptativos. Por ejemplo, se esperaría que, en sitios con alta estacionalidad térmica, los individuos presentaran termorregulación conductual activa y que las especies que viven en sitios cálidos serán más tolerantes al calor que aquellas especies que viven en sitios fríos (y viceversa).

Para poner a prueba la adaptación de los phrynosomatidos a su ambiente térmico, mediante el método poblacional, un buen modelo de estudio sería una población que habite un sitio en el que la temperatura ambiental cambie significativamente a través del tiempo (por ejemplo, entre estaciones o años). Para poner a prueba la adaptación de los phrynosomatidos a su ambiente térmico, mediante una comparación interespecífica, es necesario elegir a poblaciones que en conjunto habiten ambientes térmicos contrastantes (por ejemplo, gradientes altitudinales y latitudinales). En el mejor escenario, si los ajustes conductuales de los phrynosomatidos les permiten mantener con efectividad su T_b dentro de su T_{pref}, y además su CT_{min}, su T_{pref} y su CT_{max} cambian de forma correlacionada (positivamente) con el ambiente térmico, entonces se podría sugerir que los phrynosomatidos son capaces de sobrevivir y tener éxito reproductor aún ante el aumento de la temperatura ambiental por cambio climático. En el peor escenario, si los phrynosomatidos no son capaces de mantener con efectividad su T_b dentro de su T_{pref} y/o además su CT_{min}, su T_{pref} y su CT_{max} no cambian de forma correlacionada (positivamente) con el ambiente térmico, entonces se podría sugerir que son susceptibles al cambio climático.

En el capítulo uno (termorregulación conductual y plasticidad de la fisiología térmica en dos poblaciones de lagartijas) de la presente tesis se eligieron como modelos de estudio a dos poblaciones de dos especies. La primera fue una población de la lagartija espinosa *Sceloporus torquatus* que habita en el Jardín Botánico de Ciudad Universitaria, UNAM. En el sitio de estudio la temperatura ambiental cambia significativamente a través de los meses, lo cual fue ideal para estimar mensualmente (de mayo 2016 a abril 2017) la efectividad en la termorregulación, la CT_{min}, T_{pref} y CT_{max} de los individuos e inferir plasticidad fisiológica. Se encontró que los individuos mantienen con efectividad su T_b dentro de su intervalo de T_{pref} a pesar de la variación diaria y mensual de la temperatura ambiental. Además, el promedio mensual poblacional de la CT_{min} y la T_{pref} cambia de forma correlacionada (positivamente) con la temperatura ambiental, con lo cual se infirió plasticidad fisiológica de esos dos atributos fisiológicos. Derivado de ese trabajo fue publicado el artículo de requisito en el *Journal of Thermal Biology* (2019) titulado: *Interactions between thermoregulatory behavior and physiological acclimatization in a wild lizard population*. La segunda fue una población de la lagartija cornuda de montaña *Phrynosoma orbiculare* que habita en la periferia de un bosque de pino-encino en el municipio de Cuautepec de Hinojosa, estado de Hidalgo. En el sitio de estudio la temperatura ambiental promedio en el año 2019 fue ~1°C más caliente que en el año 2018, lo cual permitió evaluar a través de un método de marcaje-recaptura si la CT_{min}, T_{pref} y CT_{max} de los individuos aumentó mediante plasticidad fisiológica de un año a otro. Se encontró que en el año más caliente (2019), los individuos presentaron menor tolerancia al frío (menor CT_{min}) y preferencias por temperaturas más altas (mayor T_{pref}) en comparación con el año previo, demostrando plasticidad fisiológica de esos dos atributos. Derivado de ese trabajo fue publicado un artículo en el *Jornal of Experimental Zoology*

Part A (2020) titulado: *Thermal physiology responds to interannual temperature shifts in a montane horned lizard*, *Phrynosoma orbiculare*.

En el capítulo dos (evolución fisiológica y de historias de vida en una familia de lagartijas), mediante trabajo de campo y revisión de literatura, se obtuvo información de efectividad de la termorregulación conductual y características de la fisiología térmica (CT_{min}, T_b, T_{pref} y CT_{max}) para 104 especies de phrynosomatidos, que en conjunto habitan sitios con temperatura anual promedio (*annual mean temperature*; bio1) de 1°C hasta los 28°C. Se documentó que en cualquier ambiente térmico los individuos mantienen con efectividad su T_b dentro de su intervalo de T_{pref}. Con respecto a las características de la fisiología térmica, se encontró un efecto del modo de paridad, ya que las especies vivíparas tienen menor CT_{min}, T_b, T_{pref} y CT_{max} que las especies ovíparas. La CT_{min} de especies ovíparas y vivíparas cambia de forma correlacionada (positivamente) con la temperatura ambiental, la T_b y la T_{pref} cambia de forma correlacionada (positivamente) con la temperatura ambiental en especies ovíparas, pero no en especies vivíparas y la CT_{max} no cambia de forma correlacionada con la temperatura ambiental en especies ovíparas ni en especies vivíparas. Debido a que las especies vivíparas mantienen conductualmente temperaturas corporales y preferidas bajas, independientemente del sitio en el que habitan, se decidió explorar la relación entre fisiología térmica y modo de paridad a través de aspectos de teoría metabólica y de historias de vida. La tasa metabólica escala con la masa e incrementa exponencialmente con la temperatura corporal[23,24]. A su vez, la tasa metabólica determina la energía destinada a la producción reproductiva (número y tamaño de las crías)[24,25]. Para integrar aspectos de teoría metabólica y de historias de vida dentro del presente estudio se recopiló información (propia y de literatura) de tamaño y masa de hembras adultas y de fecundidad anual y tamaño y masa de los neonatos. Con esa información se modeló matemáticamente la tasa metabólica y la producción reproductiva de las especies de estudio. Se obtuvo una base de datos final de 125 especies, lo cual representa el 77% de los phrynosomatidos. Con la base de datos se realizaron comparaciones entre especies por modos de paridad y se documentó que las especies vivíparas presentan una tasa metabólica baja, producto de sus preferencias por temperaturas corporales bajas. Además, esa tasa metabólica baja estuvo asociada con una menor producción reproductiva de las especies vivíparas, producto de una menor fecundidad anual. Derivado de ese trabajo, se tiene en revisión en la revista *Nature Communications* un artículo titulado: *Exceptional parallelism characterize the evolutionary transition to live birth in phrynosomatid lizards*.

OBJETIVOS

General

Poner a prueba si las lagartijas de la familia Phrynosomatidae presentan adaptación conductual y de la fisiología térmica (en temperatura corporal preferida (T_{pref}), tolerancia al frío (CT_{min}) y tolerancia al calor (CT_{max})) a la temperatura ambiental, mediante estudios poblacionales e interespecíficos, lo cual nos permitirá inferir mecanismos de respuesta de ectotermos al cambio climático.

Particulares

1. Poner a prueba termorregulación conductual y plasticidad de la fisiología térmica (en T_{pref}, CT_{min} y CT_{max}) en dos poblaciones de lagartijas de la familia Phrynosomatidae: *Sceloporus torquatus* y *Phrynosoma orbiculare*.

2. Poner a prueba mediante el método comparativo filogenético si las lagartijas de la familia Phrynosomatidae presentan adaptación conductual y de la fisiología térmica (en T_{pref}, CT_{min} y CT_{max}) al ambiente térmico donde habitan.

CAPÍTULO 1. TERMORREGULACIÓN CONDUCTUAL Y PLASTICIDAD DE LA FISIOLOGÍA TÉRMICA EN DOS POBLACIONES DE LAGARTIJAS

1.1. *Interactions between thermoregulatory behavior and physiological acclimatization in a wild lizard population*

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Interactions between thermoregulatory behavior and physiological acclimatization in a wild lizard population

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Abreviations:
Ct_{max}Critical thermal maximum
Ct_{min}Critical thermal minimum
db¯Accuracy of body temperatures
de¯Thermal quality of the habitat
*E*Effectiveness of temperature regulation
*ET*Environmental air temperature
ET_{act}Environmental temperature of the activity period
ET_{ina}Environmental temperature of the inactivity period
T_bBody temperature
T_eOperative temperatures
T_{pref}Preferred body temperature

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Preferred body temperature

ABSTRACT

Although the importance of thermoregulation and plasticity as compensatory mechanisms for climate change has long been recognized, they have largely been studied independently. Thus, we know comparatively little about how they interact to shape physiological variation in natural populations. Here, we test the hypothesis that behavioral thermoregulation and thermal acclimatization interact to shape physiological phenotypes in a natural population of the diurnal lizard, *Sceloporus torquatus*. Every month for one year we examined thermoregulatory effectiveness and changes in the population mean in three physiological parameters: cold tolerance (Ct_{min}), heat tolerance (Ct_{max}), and the preferred body temperature (T_{pref}), to indirectly assess thermal acclimatization in population means. We discovered that *S. torquatus* is an active thermoregulator throughout the year, with body temperature varying little despite strong seasonal temperature shifts. Although we did not observe a strong signal of acclimatization in Ct_{max}, we did find that Ct_{min} shifts in parallel with nighttime temperatures throughout the year. This likely occurs, at least in part, because thermoregulation is substantially less effective at buffering organisms from selection on lower physiological limits than upper physiological limits. Active thermoregulation is effective at limiting exposure to extreme temperatures during the day, but is less effective at night, potentially contributing to greater plasticity in Ct_{min} than Ct_{max}. Importantly, however, T_{pref} tracked seasonal changes in temperature, which is one the factors contributing to highly effective thermoregulation throughout the year. Thus, behavior and physiological plasticity do not always operate independently, which could impact how organisms can respond to rising temperatures.

1. Introduction

The contemporary rate of environmental warming presents an unprecedented challenge to organisms worldwide (Barnosky et al., 2011). Understanding how climate warming will impact natural populations is a central imperative for scientists and conservation managers alike (Bellard et al., 2012; Thomas et al., 2004). This goal is especially relevant for ectotherms because their physiology and life history are highly sensitive to temperature change (Deutsch et al., 2008; Huey and Tewksbury, 2009; Huey et al., 2009). Some forecasts, for example, predict massive global declines in lizard populations over the next century, largely due to the tight connection between environmental temperature, physiological performance, and activity times in these organisms (Sinervo et al., 2010). Yet, some biological impacts of climate warming can be mitigated by the organisms themselves through short-time responses, namely through behavioral adjustments and physiological plasticity (Huey et al., 2012; Kearney et al., 2009; Seebacher et al., 2015; Sunday et al., 2014).

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Ectotherms may use behavioral tactics to compensate for the effects of thermal variation and, thus, maintain body temperatures (T_b) within their preferred ranges, even in the face of rising temperatures (Kearney et al., 2009; Sunday et al., 2014). For example, individuals may preferentially select thermally suitable microhabitats, shuttle between sun and shade, or retreat to thermal refuges when environmental conditions become too hostile (Cowles and Bogert, 1944; Kearney et al., 2009; Kirchhof et al., 2017; van den Berg et al., 2015). In addition to behavioral adjustments, ectotherms may physiologically respond to environmental warming through plastic shifts in their thermal requirements or their thermal tolerances (Seebacher et al., 2015). In response to changes in thermal environment, for example, reptiles can often produce both short- and long-term acclimation responses to better match their physiological performance to the local thermal conditions (Gunderson and Stillman, 2015; Pintor et al., 2016; Seebacher, 2005).

Importantly, the ability for organisms to plastically remodel their physiology or to alter their behavioral strategies are unlikely to operate in isolation. Rather, behavioral thermoregulation and physiological plasticity are likely to interact (Huey et al., 2012; Williams et al., 2008). Specifically, regulatory behaviors (e.g., thermoregulation) effectively buffer organisms from environmental variation, thus precluding the need to shift their physiology (and physiological plasticity) even in the face of environmental change, a phenomenon known as the Bogert effect (Huey et al., 2003). As such, physiological traits that may be behaviorally buffered should exhibit less plasticity than traits that behavior cannot as effectively shield from thermal variation (Marais and Chown, 2008). For example, lizards can behaviorally mitigate high temperatures in the daytime (when thermal heterogeneity is high, allowing for behavior to be effective), thus buffering their upper physiological limits from selection (Huey et al., 2003; Muñoz et al., 2014; Muñoz and Bodensteiner, In press). In contrast, lizards cannot as effectively behaviorally buffer the thermal environment at night because temperatures are more stable, resulting in stronger selection on lower physiological limits (Ghalambor et al., 2006; Muñoz et al., 2014). As such, physiological plasticity on upper limits should be limited by behavioral thermoregulation in the daytime, whereas the ineffectiveness of behavioral thermoregulation during the night should result in greater plasticity in lower physiological limits. In some cases, however, behavioral thermoregulation and physiological compensation can work synergistically: for example, some reptiles behaviorally select lower body temperatures in cold months, even when environmental conditions do not constrain thermoregulation (Firth and Belan, 1998). Thus, the potential interactions between behavioral flexibility and physiological compensation are likely complex, and illuminating these interactions can greatly improve our knowledge of how vulnerable organisms will be to environmental warming (Kearney et al., 2009; Seebacher et al., 2015). In general, examinations of behavioral flexibility have been conducted in field settings, whereas physiological plasticity has largely been examined in the laboratory through acclimation experiments in which plastic shifts are induced in response to set temperature change in a controlled thermal environment (Franklin et al., 2007; Kaufmann and Bennett, 1989; Kolbe et al., 2012; Pintor et al., 2016). However, studies simultaneously examining both behavior and plasticity in a natural setting are currently lacking (Nielsen and Papaj, 2017).

The goal of our study was to simultaneously examine the connections between behavioral thermoregulation and physiological plasticity induced by seasonal environmental fluctuations in natural populations of animals (thermal acclimatization). We focused our study on a population of the spiny lizard, *Sceloporus torquatus*, that inhabits xerophytic scrub in Central Mexico, where air temperatures fluctuate both daily and seasonally (Castillo Argüero et al., 2007; Página PEMBU, 2017). We note that "seasonal" is a relative term, and that seasonality at this site, though present, does not occur at the scale or magnitude of more temperate latitudes. *Sceloporus torquatus* is a boulder- and rock-dwelling lizard, which tends to bask during daytime hours and retreat to crevices at night (Jiménez Arcos, 2008). *Sceloporus* lizards are well-recognized thermoregulators (Lara-Reséndiz et al., 2014), which (in addition to the fluctuating thermal conditions of its habitat) make them an ideal organism for studying the interactions between behavioral buffering and physiological plasticity in a natural setting.

We address two specific hypotheses in this study. First, we predicted that thermoregulation allows diurnal lizards to behaviorally buffer extreme temperatures during daytime hours, thus maintaining a relatively constant body temperature throughout the year. Second, we predicted that the behavioral thermoregulation limits physiological plasticity for upper thermal limits, but not lower thermal limits. Specifically, we predicted that diurnal thermoregulatory efficiency should remain constant (or nearly so) throughout the year, resulting in limited physiological plasticity in heat tolerance and the preferred temperature. In contrast, cold tolerance should shift throughout the year in parallel with local temperatures, such that lizards should be more cold tolerant during winter than during summer.

2. Material and methods

2.1. Field sites and measurement of environmental temperatures

Field work was conducted from May 2016 to April 2017 in "Reserva Ecológica del Pedregal de San Ángel" (REPSA; 19° 19′ 8.8″ N, 99° 11′ 36.4″ W, elevation 2320 m), Mexico City, Mexico. The vegetation of study site is characterized by xerophytic scrub and the climate is subhumid temperate with a mean annual temperature of 15.6 °C (Castillo Argüero et al., 2007). The soil of the study site is covered with solidified lava (Xitle volcano) and crevices in the volcanic rocks provide refuges for the lizards (Feria Ortiz et al., 2001). Our study focused on adults of *Sceloporus torquatus* (> 73 mm snout-vent-length [SVL] in females and > 70 mm SVL in males; Feria Ortiz et al., 2001). During two consecutive days of each month for one year, four people did field work from 9:00–18:00 (activity period of lizards) and captured active (perching) lizards by noose. The field body temperature (T_b) was measured within 10 s of capture by inserting a thermocouple (Type K) connected to digital thermometer (Fluke 51-II ®) approximately 5 mm into the lizard's cloaca. Concurrent with the two days of behavioral observations per month, we also recorded operative temperatures (T_e, which represents the equilibrium temperature of an animal in the absence of physiological temperature regulation) (Bakken, 1992). By creating a null distribution of operative temperatures, we could determine the effectiveness of thermoregulation during the same period (Hertz et al., 1993). T_e´s were recorded using ten previously field-calibrated pipe models (polyvinylchloride; PVC) painted with gray spray paint. Into each model we inserted one temperature data logger (Thermochron iButton®; DS1921G), which recorded temperature every 10 min during the activity period of lizards. The models were similar in shape and size (10 cm of length and 20 mm of diameter) with respect to *S. torquatus*. The temperatures of the pipe model are strongly correlated with body temperatures of the lizards (Pearson´s correlation, $r = 0.94$, $P < 0.001$). The pipe models were placed randomly in ten semi-lit microsites occupied by lizards following Lara-Reséndiz et al. (2015). Concurrent with the two days of behavioral observations per month, we recorded the operative temperatures during the inactivity period of lizards (T_e night).

For the experimental physiological procedures (see Section 2.2 for details) the individuals were transported to the laboratory (Laboratorio de Herpetología2, Instituto de Biología-UNAM, located ~300 m from the study site).

To know the environmental air temperature (*ET*) of the xerophytic scrub throughout the year (i.e., not just on T_e sampling days), we downloaded weather station data (April 2016-April 2017). This database registered air temperature every 30 min from a weather station "CCH S" located ~ 1 km away from the study site (Página PEMBU, 2017). The downloaded environmental temperatures were separated by time of day, with the 'activity period' represented by 9:00–18:00 (ET_{act})

and the 'inactivity period' represented by 18:00–9:00 (ET_{ina}). We averaged data of ET for the whole year. To test for differences in ET among the months of year we performed Kruskal-Wallis one-way tests. We also averaged ET for each month (the mean of previous ~30 days for field work period). Thus, whereas operative temperature (T_e) data were collected during the same days as field sampling with the purpose of calculating the effectiveness of thermoregulation, environmental temperatures (ET) were collected throughout the year for the purpose of correlating with physiological traits. Our motivation for collecting both T_e and ET is that behavioral traits tend to vary with local weather conditions (discussed in Angilletta, 2009), whereas physiological traits tend to shift in parallel with weekly and seasonal trends (Phillips et al., 2016).

2.2. *Estimates of preferred body temperatures and critical temperatures*

Within a day of field collection, we estimated the preferred body temperature (T_{pref}) of lizards (Hertz et al., 1993) by placing them within a laboratory thermal gradient during their activity period (9:00–18:00). This trait is used to calculate the effectiveness of thermoregulation (described in Section 2.3). The preferred temperature was estimated every hour during this experiment, and we measured the T_b using the same digital thermometer used in the field work. The laboratory thermal gradient consisted of a wooden shuttle box (dimensions: 100 cm long, 100 cm wide, and 30 cm tall) divided into ten tracks with insulation barriers to prevent behavioral interactions among lizards (Lara-Reséndiz et al., 2015) and filled with 1–2 cm of Peat Moss. The wooden shuttle box was housed in the laboratory at an ambient temperature of ~20 °C. We placed one bulb of 90 W into each track at a height of 25 cm to generate a thermal gradient ranging from ~20 °C to ~50 °C. During the first two months of the study (May and June) we measured snout-vent length (SVL) and weight of every individuals to determine whether body size or mass affected their physiological traits. We performed Pearson´s correlation analysis and did not find any correlation between SVL and T_{pref} ($r = -0.06$, $P > 0.05$) or between weight and T_{pref} ($r = -0.07$, $P > 0.05$). Thus, for the remaining ten months of the experiment, we did not measure body size and mass.

After the laboratory thermal gradient experiment, we estimated the critical thermal maximum (Ct_{max}) once per individual and, on the following day, the critical thermal minimum (Ct_{min}) once. The Ct_{max} and Ct_{min} are the upper and lower temperatures, respectively, at which organisms loses locomotion, thus representing the viable thermal range for motion (Huey, 1982). Following established methods (Muñoz et al., 2014; Spellerberg, 1972), we estimated heat and cold tolerance, as described below.

So as to avoid inducing detrimental effects on embryos (Beuchat, 1986), we did not include gravid females in the thermal limits experiments. To estimate Ct_{max} we placed each lizard into a circular plastic container (25 cm diameter and 30 cm height) and increased their T_b at a rate of 1 °C/min by exposing them to a 100 W bulb suspended 30 cm above the container. We began flipping lizards onto their back when we observed panting behavior (Moreno Azócar et al., 2013). To estimate Ct_{min} we placed each lizard into a small plastic container (dimensions: 15 cm long, 15 cm wide, and 5 cm tall). The plastic container was placed into an ice box filled with 10 cm of crushed ice and each 30 s we flipped the lizard until failed to right itself in the next 15 s (Muñoz et al., 2014; Spellerberg, 1972). In both experiments we registered the lizard´s body temperature by inserting ~1 cm of a thermocouple into the lizard´s cloaca when the lizard could not right itself when is flipped onto its back. Finally, after these experiments all lizards were fed and hydrated ad libitum and released back at their capture sites. To test whether sex impacts T_{pref}, Ct_{max}, or Ct_{min} we performed *t*-tests with data from May and June. We did not find any significant effects of sex for T_{pref} ($t = 0.2$, $P > 0.05$), for Ct_{max} ($t = 0.4$, $P > 0.05$) or for Ct_{min} ($t = 1.9$, $P > 0.05$). Thus, we combined male and female data for our analyses.

During the first three months of the study we marked lizards using non-toxic paint. We never re-captured any of these individuals in subsequent months, although the shedding period meant some of the lizards could have lost their marking. Given the large sampling site (several hundred square meters) and the large population densities *Sceloporus* lizards achieve (Degenhardt et al., 1996), we suspect that most if not all individuals were not resampled during the study. In the unlikely case that we did resample an individual, the transient impacts of thermal tolerance trials (i.e., hardening) are unlikely to impact our results, especially given the long spacing between experimental days.

2.3. *Effectiveness of behavioral thermoregulation*

Thermoregulation occurs when organisms use behavior to maintain a certain mean body temperature (Cowles and Bogert, 1944; Huey, 1982). The effectiveness (E) of thermoregulation estimates how well organisms maintain their body temperature (T_b) within their preferred thermal range (T_{pref}), given the operative temperatures (T_e) available in their habitat. We estimated the effectiveness (E) of temperature regulation for each month of the experiment following Hertz et al. (1993), via the following equation: $E = 1\text{-}(\bar{db}/\bar{de})$. $\bar{db}$ is the average of the accuracy of body temperatures and $\bar{de}$ is the average thermal quality of the habitat. $\bar{db}$ indicates the deviation of T_b from T_{pref}, with values close to zero 0 indicating that lizards accurately maintain temperatures within their preferred range. $\bar{de}$ indicates the deviation of T_e from T_{pref}, with values close to 0 indicating high thermal quality of the habitat (i.e., habitat naturally falls within the preferred range, or close to it). Highly efficient thermoregulators will have a low $\bar{db}$ even in habitats where operative temperatures deviate from the preferred range (high $\bar{de}$), resulting in E values close to 1. As thermoregulatory efficiency decreases, in contrast, E will approach 0.

Monthly data for field body temperature (T_b), monthly interquartile range of T_{pref} (25% and 75%; T_{pref25} and T_{pref75}, respectively) and all monthly data of operative temperatures (T_e) of the ten models were used each month to calculate the indices (Hertz et al., 1993). If T_b for each individual $<$ T_{pref25}, then db for each individual $= T_{pref25}\text{-}T_b$, and if T_b for each individual $>$ T_{pref75}, then db for each individual $= T_b\text{-}T_{pref75}$. If T_b for each individual is within interquartile range of T_{pref} then db for each individual $= 0$. If each $T_e < T_{pref25}$, then $de = T_{pref25}\text{-}T_e$., and if each $T_e > T_{pref75}$, then $de = T_e\text{-}T_{pref75}$. If each T_e is within interquartile range of T_{pref} then $de = 0$.

2.4. *Statistical analysis*

Due to differences in the distribution of the data, we tested for differences in T_b, T_{pref} and Ct_{max} through the months of year using a Kruskal-Wallis one-way tests and to tested for differences in Ct_{min} through the months of year using a one-way Analysis of Variance (ANOVA). We examined differences among months using post hoc tests. To test the influence of environmental temperature on thermal physiology (acclimatization) of *S. torquatus* we performed Pearson´s correlation analysis between environmental traits (ET_{act} and ET_{ina}) and all physiological traits (T_{pref}, Ct_{min} and Ct_{max}).

3. Results

3.1. *Environmental temperatures*

The mean of ET in the xerophytic scrub was 16.3 ± 4.6 °C s.d. We did find significant differences in mean monthly of ET ($H_{11,\ 17514} = 1599.2$, $P < 0.001$; Fig. 1A). Monthly mean of ET ranged from 14.2 °C to 19 °C, with the monthly mean of ET_{act} ranging from 18.5 °C to 23.5 °C and the monthly mean of ET_{ina} ranging from 11.2 °C to 16 °C (Table 1). Thus, the monthly means ranged roughly 5 °C across seasons both during the day and at night, and nights were substantially colder than daytime temperatures. Nonetheless, the mean temperature of

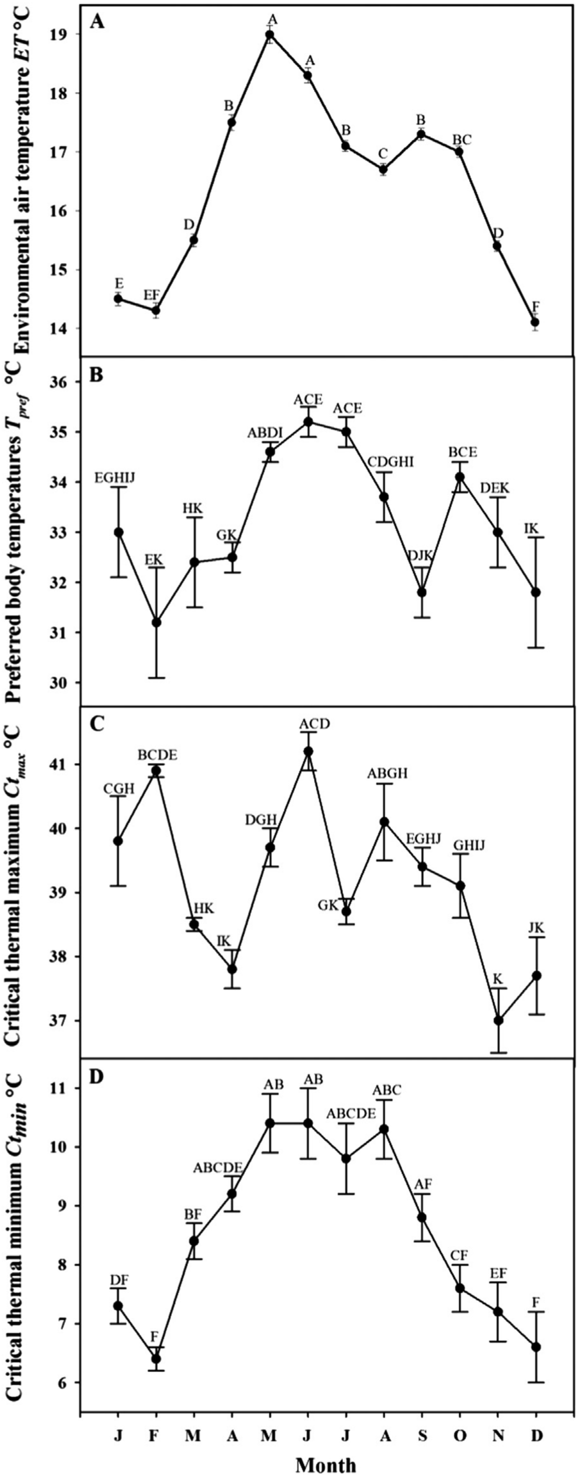

Fig. 1. A. Monthly environmental air temperature (*ET*) at Reserva Ecológica del Pedregal del San Ángel. B. Monthly preferred body temperatures (T_{pref}) of *Sceloporus torquatus*. C. Monthly critical thermal maximum (Ct_{max}) of *Sceloporus torquatus*. D. Monthly critical thermal minimum (Ct_{min}) of *Sceloporus torquatus*. Letters represent significant differences and bars represent ± 1 s.e.m.

activity periods were substantially cooler than observed body temperatures, indicating that, throughout the year, lizards were targeting a warmer subset of environmental temperatures. The monthly mean of operative temperatures in the day (T_e) registered at xerophytic scrub ranged from 15 °C to 28.2 °C and was highly variable compared with the monthly mean of operative temperatures registered at night (T_e night), which ranged from 6.6 °C to 14.7 °C (Table 1). Overall, T_e ranged from − 0.5–63 °C throughout the year (Fig. 2).

3.2. Summary of body temperatures and physiological traits

We collected body temperatures from 5 to 19 *S. torquatus* individuals every month for a year. The monthly mean of T_b ranged less than 4 °C across the year, ranging from 31.1 °C in June to 34.9 °C in May (Table 1). Individual T_b ranged from 27.1 °C to 38.5 °C throughout the year (Fig. 2). The monthly mean of preferred body temperatures (T_{pref}) of *S. torquatus* ranged from 31.2° to 34.6°C (Table 1). The monthly mean of Ct_{min} ranged from 6.4° to 10.4°C and the mean of Ct_{max} ranged from 37° to 41.2°C (Table 1).

3.3. Effectiveness of behavioral thermoregulation

The monthly effectiveness of temperature regulation (*E*) of *S. torquatus* remained high throughout the year, ranging from 0.71 to 1. This occurred through high behavioral accuracy, as ($\bar{db}$) remained low throughout the year, ranging from 0 to 3.2 despite relatively poor thermal habitat quality ($\bar{de}$ ranged from 5.4 to 18.1) (Table 2). The T_{pref25} ranged from 25.8° to 34.2°C and the T_{pref75} ranged from 34.3° to 36.8°C (Table 2).

3.4. Statistical analyses comparing environment to physiology

We did not find any significant differences in mean monthly T_b throughout the year ($H_{11,\ 118}$ =13.8, P = 0.25). However, we did find significant differences in mean monthly T_{pref} ($H_{11,\ 118}$ =31.6, P < 0.001; Fig. 1B), Ct_{max} ($H_{11,\ 99}$ =44.8, P < 0.001; Fig. 1C) and Ct_{min} ($F_{11,\ 99}$ =8.25, P < 0.001; Fig. 1D). Thus, in addition to the linear correlations with *ET* described below, there were month-to-month differences in mean population variables.

We found positive correlations between ET_{ina} and T_{pref} (r = 0.7, P = 0.01; Fig. 3a) and between ET_{ina} and Ct_{min} (r = 0.84, P < 0.001; Fig. 3b), but we did not find a correlation between ET_{ina} and Ct_{max} (r = 0.18, P = 0.6; Fig. 3c) or between ET_{act} and Ct_{max} (r = 0.27, P = 0.4; Fig. 3d).

4. Discussion

In the face of rising temperatures, ectotherms such as lizards may respond through behavioral changes, physiological plasticity, or through a combination of these two features (Huey et al., 2012; Kearney et al., 2009; Sunday et al., 2014). The goal of our study was to determine whether and how behavioral thermoregulation and physiological plasticity interact in a natural population of *Sceloporus torquatus*, and consider how these interactions might shape their vulnerability to climate change. Our results suggest that thermoregulatory behavior is highly effective at buffering *S. torquatus* from thermal variation. Over the course of the year of this study, we found dramatic differences in thermal environment (observed daily range in operative temperatures exceeding 40 °C), and mean environmental temperatures ranging from 11.2 °C (at night in winter) to 23.5 °C (during the day in spring). Despite living in both seasonally and diurnally variable habitats, lizards were remarkably efficient at actively targeting a relatively narrow range of body temperatures (~31.1–34.9 °C) during the day, resulting in near-perfect thermoregulatory effectiveness (*E* close 1) during their active period throughout the year.

Due to behavioral thermoregulation *S. torquatus* maintained relatively constant T_b, throughout the year. This result supports our first hypothesis that behavioral thermoregulation allows diurnally active lizards to avoid environmental extremes in the daytime. The maintenance of T_b is relevant because of the tight relationships between body temperature, whole-organism performance and fitness of ectotherms (Angilletta et al., 2002). The ability to effectively

Table 1

Monthly mean of field body temperatures (T_b), preferred body temperature (T_{pref}), critical temperatures (Ct_{min} and Ct_{max}), operative temperatures in the day (T_e) and at night (T_e night) and environmental temperatures (*ET*) in the activity and the inactivity period (ET_{act} and ET_{ina}) of *Sceloporus torquatus* in a xerophytic scrub in Mexico City, Mexico. Within parenthesis we show ± standard deviation. *N* indicates the sample size and N_c indicates the sample size used for critical temperatures.

Month	*N*	N_c	T_b	T_{pref}	Ct_{min}	Ct_{max}	T_e	T_e night	*ET*	ET_{act}	ET_{ina}
J	7 (2♀,5♂)	6	34.3(1.5)	33(6)	7.3(0.9)	39.8(1.8)	18.3 (7.9)	6.6 (3.7)	14.5(4.6)	18.7(3.6)	11.8(2.8)
F	5 (2♀,3♂)	3	34(2.1)	31.2(6.2)	6.4(0.5)	40.9(0.2)	23.3 (8.9)	9.4 (4.3)	14.3(5.1)	18.9(3.5)	11.2(3.3)
M	7 (4♀,3♂)	3	33.6(2)	32.4(5.4)	8.4(0.6)	38.5(0.1)	28.2 (11.9)	11.2 (5.4)	15.5(4.8)	19.9(3.4)	12.6(3.2)
A	19 (13♀,6♂)	10	34(2)	32.5(4.1)	9.2(1.1)	37.8(0.9)	27.8 (8.3)	14.7 (4)	17.5(5.1)	22.5(3.2)	14.3(3.2)
M	11 (6♀,5♂)	10	34.9(2.4)	34.2(2.8)	10.4(1.7)	39.7(0.9)	27.3 (7.2)	13.2 (2.8)	19(5.1)	23.5(3.8)	16(3.5)
J	10 (5♀,5♂)	10	31.1(3.7)	34.6(2.8)	10.4(1.8)	41.2(1.1)	23.4 (5.8)	12.9 (2.9)	18.3(4.4)	22.2(3.5)	15.7(2.6)
J	10 (5♀,5♂)	10	34.2(3.2)	34.5(2.4)	9.8(1.7)	38.7(0.8)	21.5 (4.7)	13.7 (2.2)	17.1(3.7)	20.7(2.6)	14.7(2)
A	11 (4♀,7♂)	10	31.9(3)	33.4(4.6)	10.3(1.8)	40.1(1.9)	20.3 (3.6)	14.6 (2.1)	16.7(3.6)	20.7(2.6)	14.4(1.9)
S	9 (4♀,5♂)	9	33.7(2.8)	31.7(4.1)	8.8(1.2)	39.4(0.7)	21.1 (5.6)	13.8 (2.4)	17.3(3.6)	20.8(2.7)	15(2)
O	11 (3♀,8♂)	11	33.1(2)	34.4(2.5)	7.6(1.3)	39.1(1.6)	21.3 (5.2)	13.6 (2.2)	17(3.6)	20.5(2.8)	14.8(1.9)
N	9 (5♀,4♂)	8	32.9(3.9)	33.1(3.3)	7.2(1.3)	37 (1)	15 (2.1)	12.6 (1.2)	15.4(3.8)	18.5(3.4)	13.4(2.3)
D	9 (2♀,7♂)	9	32.8(2.6)	31.8(6.2)	6.6(1.9)	37.7(1.4)	18 (4.9)	14.6 (1.7)	14.2(4.8)	18.5(3.8)	11.3(2.8)

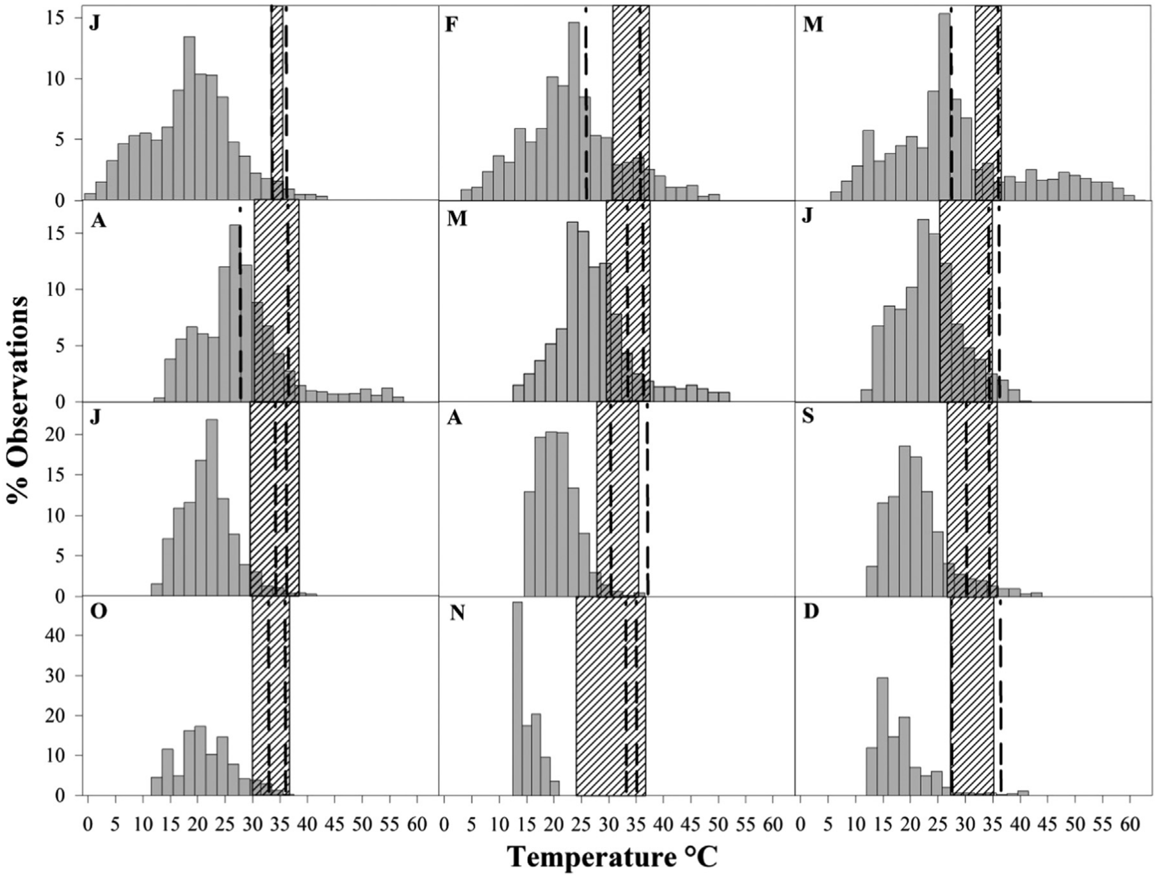

Fig. 2. Monthly body temperatures (T_b) and preferred body temperatures (T_{pref}) of *Sceloporus torquatus* and operative temperatures (T_e) at Reserva Ecológica del Pedregal del San Ángel. The diagonal shading represents the monthly range of T_b, the dashed lines represent the monthly interquartile range of T_{pref} and vertical bars represent the observations of T_e.

thermoregulate is certainly not limited to *S. torquatus*. Indeed, active thermoregulation is typical of many other lizards (including other phrynosomatids) (Artacho et al., 2017; Díaz de la Vega-Pérez et al., 2013; Hertz et al., 1993; Kirchhof et al., 2017; Lara-Resendiz et al., 2013, 2014, 2015; Muñoz et al., 2016; Muñoz and Losos, 2018; Ortega et al., 2016; Sartorius et al., 2002), amphibians (Balogová and Gvoždík, 2015; Gvoždík and Kristín, 2017; Strickland et al., 2016) and insects (Kearney et al., 2009; Kingsolver, 1983; Sunday et al., 2014), to give a few examples. The stability in thermoregulatory efficiency that we observe in *S. torquatus* may be a relatively widespread ectotherm mechanism for buffering environmental variation.

In the face of rising environmental temperatures, active thermoregulation has a relevant role for the ectotherms avoiding detrimental temperatures (Kearney et al., 2009; Sunday et al., 2014). For example, when the environmental temperature is high, *Sceloporus* lizards may retreat to cool thermal refuges to avoid encountering lethal temperatures (Sinervo et al., 2010). We do not address the proximate mechanisms facilitating thermoregulation in this study (i.e., relative sun/shade use, structural microhabitat use), but note that if the availability of preferred microsites is eroded due to warming, so too will the ability to thermoregulate. Nonetheless, behavioral efficiency is likely constrained by fundamental limits: below a minimum amount of activity, organisms cannot grow or maintain their body condition. If constraints on activity persist, population size may decrease over time

Table 2
Monthly interquartile range of preferred body temperature (T_{pref}) (25% and 75%; T_{pref25} and T_{pref75}), accuracy of body temperatures ($\bar{d}b$), quality thermal of the habitat ($\bar{d}e$) and effectiveness of temperature regulation (E) of *Sceloporus torquatus* in a xerophytic scrub in Mexico City, Mexico.

Month	T_{pref25}	T_{pref75}	$\bar{d}b$	$\bar{d}e$	E
J	33.2	36	0	15.1	1
F	25.8	35.7	0.3	7.2	0.96
M	27.6	36.3	0.03	6.4	0.99
A	29.9	35.6	0.2	5.4	0.97
M	33.3	36	0.7	7.8	0.91
J	34.2	36	3.2	10.9	0.71
J	33.9	35.9	1.7	12.4	0.86
A	30.1	36.8	0.7	9.8	0.93
S	30.1	34.3	0.8	9.5	0.91
O	33.1	35.9	0.9	11.9	0.92
N	33.1	34.9	1.8	18.1	0.9
D	27.2	36.4	0.01	9.3	1

and, eventually, go extinct (Sinervo et al., 2010, 2011). Thus, while behavioral efficiency may be effective in the short run – and it is a clear tack employed by *S. torquatus* over the course of a year – other mechanisms such physiological plasticity are also important for ensuring that lizards are able to maintain activity as environmental constraints continue to mount.

Consistent with our hypotheses, we found that Ct_{min} is positively correlated with nocturnal environmental temperatures in *S. torquatus* (ET_{ina}; Fig. 3b), such that in the wintertime cold tolerance was ~4 °C lower than in the summer. The changes in the population mean of Ct_{min} across months correlated with changes in the monthly mean of ET_{ina} suggesting thermal acclimatization (phenotypic plasticity induced by thermal changes; Pörtner et al., 2009). We observed significant differences in Ct_{min} among seasons, such that means in this trait were similar across spring and summer, and autumn means were similar to winter means (Fig. 1D). This seasonal shift in thermal acclimatization within a single population is quite dramatic; such differences are usually observed over substantially greater environmental scales and among species (Huey and Tewksbury, 2009). This result indicates that cold tolerance is labile within *S. torquatus*, and shifts in parallel with environmental conditions throughout the year.

In contrast to cold tolerance, we found no relationship between Ct_{max} and nocturnal environmental temperatures (Et_{ina}; Fig. 3c) or daytime environmental temperature (Et_{act}; Fig. 3d) in *S. torquatus*. Ct_{max} was also variable (~4 °C, similar to Ct_{min}), but we did not find a pattern with seasonal thermal variation (Fig. 1C). The variation in Ct_{max} was somewhat surprising given that this trait is often relatively inert within populations, among populations, or even among closely related species (e.g., Cruz et al., 2005; Kellermann et al., 2012; Muñoz et al., 2014, 2016). Although Ct_{max} was uncorrelated with ET_{act} or ET_{ina}, it is possible that shifts in heat tolerance were induced by other environmental changes or physiological traits, such as precipitation regimes (Clusella-Trullas et al., 2011), evaporative water loss (Tracy et al., 2008) or to dehydration (Rezende et al., 2011).

Our results are consistent with the idea that thermoregulation is remarkably effective at buffering organisms from variation during the

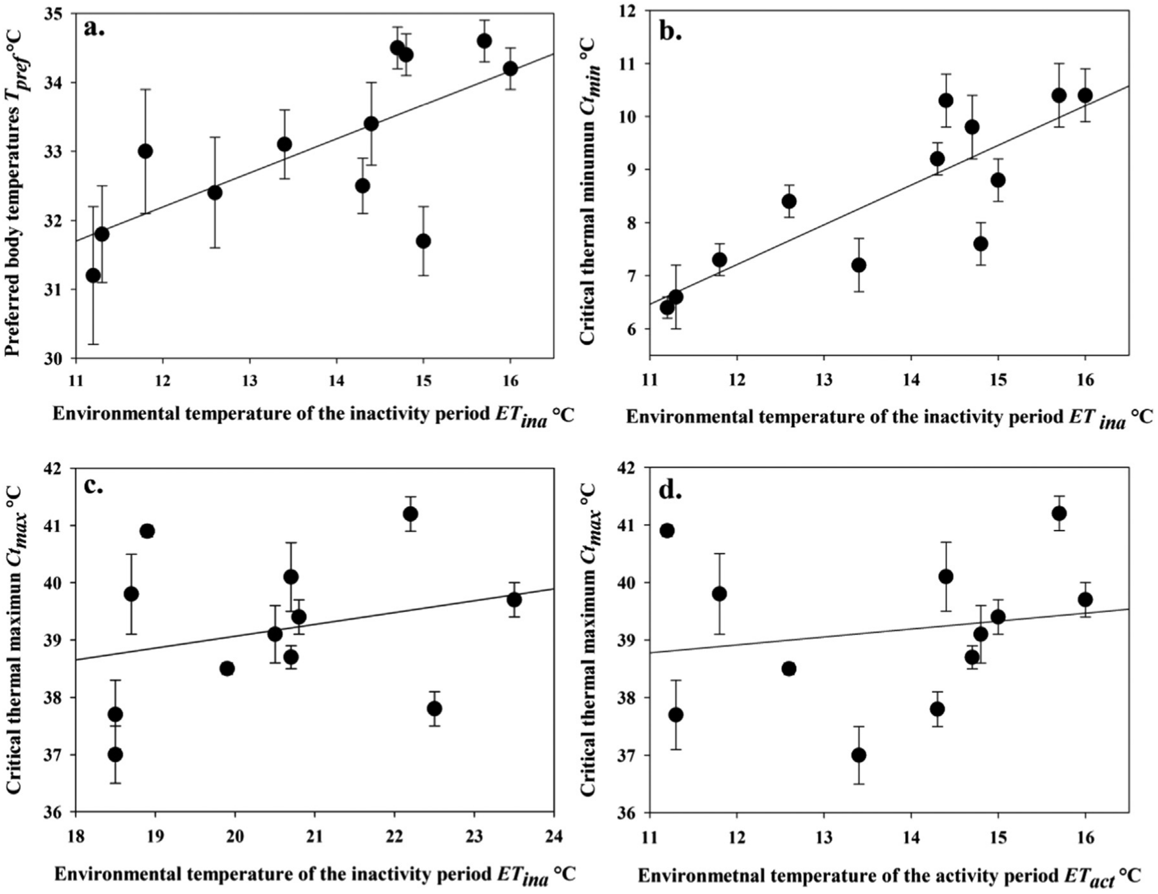

Fig. 3. a. Correlation between environmental temperature in the inactivity period of *Sceloporus torquatus* (ET_{ina}) and preferred body temperatures (T_{pref}) (r = 0.7, P = 0.01) of the lizards. b. Correlation between environmental temperature in the inactivity period of *S. torquatus* (ET_{ina}) and critical thermal minimum (Ct_{min}) (r = 0.84, P < 0.001) of the lizards. c. Weak, non-significant correlation between environmental temperature in the inactivity period of *Sceloporus torquatus* (ET_{ina}) and critical thermal maximum (Ct_{max}) (r = 0.18, P = 0.6). d. Weak, non-significant correlation between environmental temperature in the activity period of *Sceloporus torquatus* (ET_{act}) and critical thermal maximum (Ct_{max}) (r = 0.27, P = 0.4). Bars represent ± 1 se.m.

day, but not at night. Indeed, nighttime temperatures were both cooler and more stable than daytime temperatures, supporting the idea that the Bogert effect – and the behavioral regulation that underlies it – hinge on the thermal heterogeneity of the habitat (Ghalambor et al., 2006; Huey et al., 2003; Muñoz and Bodensteiner, in press). The role of behavioral thermoregulation in limiting exposure to selection on physiology has been demonstrated to dampen rates of evolution in Ct_{max} relative to Ct_{min} (Kellermann et al., 2012; Muñoz et al., 2014), and to impact geographic patterns of tolerance ranges across latitude (Araújo et al., 2013; Sunday et al., 2014; Gunderson and Stillman, 2015). Our study extends this interspecific framework to also incorporate the distinct intraspecific footprints of behavioral thermoregulation on plasticity in physiological traits within a single species.

Our findings that T_{pref} varies among months (Fig. 1B) and shifts in parallel with thermal conditions throughout the year (Fig. 3a) indicate that behavioral thermoregulation, though highly effective, is not sufficient for compensating for seasonal changes in thermal environment. During the winter *S. torquatus* targets a slightly lower temperature (~31 °C), whereas in summer it tracks higher temperatures (~34 °C). Because thermoregulatory effectiveness is contingent on the ability for organisms to maintain body temperatures within their preferred ranges, a seasonal shift in T_{pref} likely contributed to the often near-perfect estimates of effectiveness that we observed. Thus, while *S. torquatus* behaviorally thermoregulates, seasonal acclimation in the target core temperature range appears to assist in the effectiveness of this mechanism.

Our observation that physiological plasticity of T_{pref} of *S. torquatus* is correlated with ET_{ina} (Fig. 3a) is also consistent with laboratory acclimation experiments. For example, Kaufmann and Bennett (1989) experimentally treated two groups of the desert night lizard *Xantusia vigilis* at two acclimation temperatures (20 °C and 30 °C) during 50 days and they found differences in T_{pref} between groups (with the group acclimated to 30 °C have high T_{pref} than the group acclimated to 20 °C). As a caveat, however, we note that our assessments of physiological plasticity were indirect, as we did not measure the same individuals or genotypes across seasons. Without enclosures, it is challenging to track the same individuals over time, but doing so would be a fruitful future study as it would illustrate whether population-level patterns that we detected also occur within individuals across seasons.

In the face of ongoing environmental warming (IPCC, 2014), behavioral buffering and physiological plasticity are the key mechanisms that can help individuals compensate for the increase in environmental temperature (Farrell and Franklin, 2016; Franklin et al., 2007; Muñoz and Moritz 2016; Palumbi et al., 2014). Rather than working synergistically, however, behavioral thermoregulation and physiological plasticity appear to function in a complementary fashion, which should actually limit the ability for organisms to respond to rising temperatures. If thermoregulatory behavior does, indeed, contribute to limited acclimatization in upper physiological traits, then this might limit additive responses in behavior and physiological plasticity that organisms could mount. In light of this, if behavioral thermoregulation is not successful at buffering organisms from rising temperatures (discussed in Sinervo et al., 2010; Sinervo et al., 2011), we suggest that its limiting influence over thermal acclimatization in Ct_{max} could further limit the ability for ectotherms to mount sufficient responses through plasticity (Seebacher et al., 2015). In contrast, behavioral thermoregulation and plasticity in the preferred temperature do appear to interact additively, resulting in near-perfect thermoregulation throughout the year. Thus, the effects of behavioral thermoregulation on plasticity might vary between traits.

Interactions between behavioral buffering and physiological plasticity may be generally widespread. For example, color change and thermal refuge seeking behavior in butterfly larvae appear to also function non-independently, with total adaptive responses limited by their interactions (Nielsen and Papaj, 2017). We argue that disentangling whether and how behavior and plasticity interact to shape physiological variation can provide a more holistic view of the mechanisms by which ectothermic organisms will be impacted by rising environmental temperatures.

In conclusion, we propose that behavioral thermoregulation and plasticity do not operate in isolation, rather these two features interact to shape physiological phenotypes. *Sceloporus torquatus* is a near-perfect thermoregulator that maintains its body temperature within a narrow range during their activity period throughout the year. Consequently, behavioral thermoregulation allows individuals to avoid high temperatures during the day. In contrast, behavioral thermoregulation is not efficient at night, which is correlated with greater plasticity in cold tolerance. Finally, effective behavioral thermoregulation is, aided in large part by marked plastic shifts in the preferred temperature, indicating that behavior and plasticity interact to shape temporal patterns of physiological variation.

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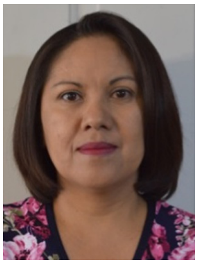

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1.2. *Thermal physiology responds to interannual temperature shifts in a montane horned lizard, Phrynosoma orbiculare*

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RESEARCH PAPER

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Thermal physiology responds to interannual temperature shifts in a montane horned lizard, *Phrynosoma orbiculare*

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Abstract

As climate change marches on, rapidly rising temperatures shatter records every year, presenting ever-growing physiological challenges to organisms worldwide. Ectotherms rely on behavioral and physiological plasticity to contend with environmental fluctuations. Nonetheless, our understanding of thermal plasticity has been largely limited to laboratory settings. Here, we test whether aspects of thermal physiology respond to interannual shifts in thermal environment in a natural population of *Phrynosoma orbiculare*, a montane horned lizard, from Hidalgo, Mexico. At our field site, 2019 was markedly warmer than the year that preceded it. We detected population-level increases in three key thermal physiological traits: preferred temperature, the critical thermal minimum, and the critical thermal maximum. Thus, thermal phenotypes appear to shift in tandem in response to environmental fluctuations. A subset of individuals were resampled across years, allowing insight into plastic shifts within an organism's lifetime. We detected parallel increases in these lizards for the preferred temperature and the critical thermal minimum, but not for the critical thermal maximum. Our results support a growing body of literature indicating that preferred conditions and cold tolerance can be highly labile over the course of an organism's lifetime, whereas hardening over shorter time periods is more common for heat tolerance. Given that heat tolerance increased at the population-level, but not in resampled individuals, it is possible that rapid evolution occurred due to temperature increases. In short, physiological shifts can be observed in natural populations over relatively short timespans, and these shifts might reflect a combination of evolutionary and acclimatory responses.

KEYWORDS
body temperature, critical thermal limits, phenotypic plasticity, physiological acclimatization, preferred body temperature, thermal physiology

1 | INTRODUCTION

Temperature is one of the most ubiquitous variables affecting biological processes (Angilletta, 2009; Gillooly, Brown, West, Savage, & Charnov, 2001; Somero, Lockwood, & Tomanek, 2017). Temperature increase due to human activities presents a global threat to biodiversity, but the magnitude of its effects is unequally distributed across the tree of life (Sala et al., 2000; Thomas et al., 2004).

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Rising temperatures present unique challenges to ectotherms such as lizards, which rely on external environment to regulate their body temperature (Huey et al., 2012; Paaijmans et al., 2013). These challenges are further magnified in montane and tropical species, as these types of organisms are especially sensitive to temperature changes and, in many cases, are already functioning near their upper physiological limits (Deutsch et al., 2008; Huey et al., 2009). Concurrently, the pace and magnitude of climate change is predicted to be particularly severe in tropical regions (Williams, Jackson, & Kutzbach, 2007). Some estimates, for example, predict massive global declines in lizard populations in this century due to rising temperatures, particularly in the tropics (Huey et al., 2009; Huey, Losos, & Moritz, 2010; Sinervo et al., 2010); when such organisms are restricted to progressively warming mountaintops, they may find themselves on an "escalator to extinction" (e.g., Freeman, Scholer, Ruiz-Gutierrez, & Fitzpatrick, 2018).

However, organisms are not exclusively at the whim and mercy of their thermal environments. Through adjustments in their behavior and/or physiological limits, organisms can compensate (at least partially) for thermal fluctuations in their environments (Huey, Hertz, & Sinervo, 2003; Huey et al. 2012; Kearney, Shine, Porter, & Wake, 2009; Moritz & Agudo, 2013; Seebacher, White, & Franklin, 2015). Many ectotherms are highly precise thermoregulators, and are able to efficiently seek out their preferred thermal conditions even under fluctuating environmental conditions (Huey, 1982; Muñoz & Losos, 2018; Seebacher, 2005; Seebacher & Franklin, 2005). Physiological acclimatization (i.e., plastic adjustments in physiological traits) can also help organisms better match their physiology to their local thermal conditions (Gunderson & Stillman, 2015; Pintor, Schwarzkopf, & Krockenberger, 2016; Seebacher, 2005). Within an organism's lifetime, for example, upper and lower critical thermal limits (CT_{max} and CT_{min}, respectively) can be higher during the summer than the winter for many lizard species (Ballinger, Hawker, & Sexton, 1969; Larson, 1961; Smith & Ballinger, 1994; Spellerberg, 1972a, 1972b). Thus, studies addressing how thermal biology responds to environmental fluctuations are of key conservation interest, but studies focused on variation in natural populations remains relatively scant (Chevin, Lande, & Mace, 2010; Merilä & Hendry, 2014; Muñoz & Moritz, 2016). The previous 5 years set global records for temperatures (NOAA, National Centers for Environmental Information, 2020). This unprecedented march toward a warmer world provides an opportunity to examine whether and how organisms use behavioral and physiological adjustments to dynamically respond to changing conditions.

The goal of our study was to investigate whether thermal physiology responded to environmental temperature increases between 2018 and 2019 in a natural population of the horned lizard, *Phrynosoma orbiculare*. This Mexican lizard is restricted to montane habitats, suggesting it may be particularly vulnerable to extinction (Dubey & Shine, 2010, 2011). At our study site in Hidalgo, Mexico (elevation = 2,500 m) temperatures were 1°C warmer in 2019 than in 2018 (Table 1), providing the opportunity for behavioral and physiological adjustments in *P. orbiculare*. In late summer 2018, we measured four key thermal traits: (body temperature [T_b], the preferred body temperature [T_{pref}], the critical thermal maximum [CT_{max}], and the critical thermal minimum [CT_{min}]). These traits describe how ectotherms like lizards interact with their thermal environments (Angilletta, 2009; Huey, 1982). We then remeasured those traits 1 year later in August–September 2019. Given higher environmental temperatures in 2019, we predicted increases in all four traits. Using a mark-recapture approach, we were able to resample a subset of individuals across years. This allowed us to estimate within-individual shifts in thermal physiology to indirectly infer whether population-level physiological shifts might reflect evolution (i.e., heritable intergenerational changes), phenotypic plasticity (i.e., intragenerational changes), or some combination of both. We predicted that any population-level differences in thermal traits should also be evident in recaptured individuals. Any phenotypic shifts at the population-level not matched by shifts in recaptured individuals suggest (but do not prove) that an evolutionary change might have occurred.

2 | METHODS

2.1 | Study organism, field sites, and body temperature measurements

The mountain horned lizard, *P. orbiculare*, is a viviparous lizard species distributed across high elevations (plateaus and mountains between ~1,400 and ~3,400 m above sea level) in the Sierra Madre

TABLE 1 Mann–Whitney *U* test of differences in climatic data (minimum, mean, and maximum daily air temperatures [°C] and mean daily precipitation [mm]) between years

Climate data	Year	Mean ± *SE*	Mann–Whitney *U* value; *p* value
Mean daily temperature	2018	12.7 ± 0.17	***U* = 58,267; *p* = .003**
	2019	13.53 ± 0.16	
Mean maximum daily temperature	2018	20.07 ± 0.21	***U* = 54,478; *p* < .001**
	2019	21.39 ± 0.21	
Mean minimum daily temperature	2018	5.43 ± 0.18	*U* = 64,869; *p* = .540
	2019	5.70 ± 0.16	
Mean daily precipitation	2018	1.69 ± 0.28	*U* = 66,515; *p* = .970
	2019	1.68 ± 0.26	

Note: Data were gathered from a weather station ~4 km away from the field site in Hidalgo, Mexico. *p*-values < .05 are presented in bold font.

Oriental and Occidental in the northern and central Mexico (Sherbrooke, 2003). Our focal population is located in an induced grassland on the periphery of a pine-oak forest (20.064°N, −98.201°W; 2,500 m) in the locality of El Encinal, municipality of Cuautepec de Hinojosa, Hidalgo, México. We performed field work (with two or three people at a time) in an area of 0.06 km^2 during 3 weeks between August 6 and September 12, 2018 and during 2 weeks from August 25 to September 8, 2019. During these periods we measured body temperatures from 08:00 to 18:00 hr (activity time of lizards in the study site; Pardo-Ramírez, 2019). We manually captured individuals and immediately (<10 s after the capture) measured their cloacal temperature (body temperature; T_b) using a type K thermocouple (±0.1°C precision) connected to a digital handheld thermometer (Fluke 51-II®). Using the same thermocouple, we then measured the temperature of the substrate where lizards were observed perching (substrate temperature; T_s) and the air temperature ~5 cm above the substrate (air temperature; T_a).

2.2 | Measurement of critical thermal limits and preferred temperatures

On the same day of capture, lizards were transported to a field laboratory (~200 m from the study site with natural light and a maintained temperature of ~20°C). Following our previous work (Domínguez-Guerrero et al., 2019), we built a thermal gradient arena that consisted of a wooden box (100 cm long, 100 cm wide, and 30 cm tall), filled with 2 cm of sediment from the study site and divided into 10 tracks. At one extreme of thermal gradient, we suspended one 75 W bulb at a height of 25 cm above the gradient's ground. This resulted in a thermal gradient ranging from ~20°C on the lower end to ~50°C at the hotter end. After being given a night to rest, we placed lizards individually into arena lanes from 08:00 to 17:00 hr. At each hour mark during the experiment, we measured body temperature of lizards with the same thermocouple and digital thermometer used in field work. Lizards were hand-captured and body temperature measured as quickly as possible (<10 s). T_{pref} was estimated as the average of the central 50% of temperatures measured during this experiment (Hertz, Huey, & Stevenson, 1993).

After the T_{pref} experiment, we placed each lizard into a plastic container (25 cm diameter and 30 cm height) to estimate their critical thermal maximum, CT_{max}. This metric describes the upper limit of ectotherm locomotion (Spellerberg, 1972a). Following established methods (Muñoz et al., 2014), we placed a 90 W bulb 40 cm above the container, and steadily increased lizard's body temperature by 1°C/min (warming and cooling rates were estimated post hoc by measuring total temperature change/total experiment length). When lizards initiated evaporative cooling through panting, we began flipping them onto their back every 20 s and encouraged them to right themselves by prodding their thigh using a pair of blunt tweezers. When the lizard failed to right itself within 15 s, we recorded their body temperature as CT_{max}. Following the CT_{max} experiment, we immediately removed lizards from the heat source, cooled them down to room temperature, and offered them water ad libitum. On the next day we placed each lizard into a rectangular container (23 cm long, 16 cm wide, and 8 cm tall) to estimate their critical thermal minimum, CT_{min}. This metric describes the lower limit of ectotherm locomotion (Spellerberg, 1972a). We placed the container on a bed of ice to decrease body temperature by a rate of 1°C/min (Muñoz et al., 2014). We flipped lizards onto their backs every 20 s. We recorded CT_{min} as the temperature at which lizards failed to right themselves after 15 s of encouragement. When we finished CT_{min} experiments, we allowed lizards to return to their preferred temperature in the thermal gradient for 1 hr. Finally, we weighed (±0.01 g) and measured each lizard (snout-to-vent length [SVL] ± 1 mm), registered their sex, and recorded their age category. We considered adult females to have SVLs > 66 mm and adult males to have SVLs > 65 mm (Hernández-Navarrete, 2018). Animals with SVLs smaller than these cut-offs were categorized as juveniles. In anticipation of our follow-up field season in 2019, in 2018 we permanently marked lizards with three beads on the tail (Figure 1) following the methods of Fisher and Muth (1989). Weather station data, including precipitation mean, maximum, and minimum daily temperatures were obtained from the Servicio Meteorológico Nacional, México. These data are derived from the station AHUPB, located in in Ahuazotepec, Puebla, Mexico (20.041°N, −98.174°W), which is approximately 4 km away from the field site. We used weather station data to characterize general weather patterns between 2018 and 2019 (Servicio Meteorológico Nacional, 2020).

2.3 | Statistical analyses

For the subset of lizards that we sampled in both 2018 and 2019, we compared thermal traits using a paired *t* test performing separate tests for each trait. We also used a paired *t* test to test for differences between microenvironmental measures (T_a and T_s) between years for these resampled individuals. To test for differences in the

FIGURE 1 Male *Phrynosoma orbiculare* with individual bead tag on tail for identification at the field site during the data collection period [Color figure can be viewed at wileyonlinelibrary.com]

macroenvironmental measures (mean, maximum, and minimum daily air temperature and precipitation) we used a Mann–Whitney rank sum test because the data were non-normally distributed. To compare population means of physiological traits between years we performed a mixed linear model with lizard ID as a random effect to account for repeat measures between years. Physiological traits (T_b, T_{pref}, CT_{min}, and CT_{max}) were designated as the dependent variables, and we included log-transformed SVL and T_a as continuous predictors and year, sex, and age class were categorical predictors. The microenvironmental measures (T_a and T_s) were highly correlated ($r = .72$; $p < .0001$), therefore, we only include T_a in the model as a predictor.

3 | RESULTS

3.1 | Variation in the thermal environment between years

At our field site the mean daily air temperature and the mean maximum daily air temperature were hotter in 2019 by approximately 0.83°C and 1.32°C respectively. However, we did not observe any differences in precipitation or the mean minimum daily temperature between years (Table 1). The lowest temperatures recorded at the weather station were −5.5°C in 2018 and −5.0°C in 2019. Meanwhile the hottest temperature recorded in 2018 and 2019 were 28.5°C and 33.5°C, respectively. Thus, not only did the mean maximum daily temperature increase, but there was also a 5°C increase between the absolute hottest temperature recorded between years. Air and substrate temperatures taken at the time and location of capture were hotter in 2019 (Figure 2). Specifically, there was approximately a 4.7°C increase in mean substrate temperature between years (mean ± standard deviation [*SD*]: 2018 = 24.98 ± 5.68; 2019 = 29.65 ± 6.43; $p < .001$), and a 3.3°C increase in the air temperature (mean ± *SD*: 2018 = 21.65 ± 4.31; 2019 = 24.96 ± 3.67; $p < .001$) at the time of capture between years.

3.2 | Lizard behavior and physiology

We collected a total of 48 individuals of *P. orbiculare* across both years, of which 20 were females and 28 were males (Table S1). Populations means for all traits (body temperature, preferred temperature, heat tolerance, and cold tolerance) were higher in 2019 than in 2018 (Table 2 and Figure 3). At the population-level the largest magnitude increase between years was in mean field measured body temperature and preferred body temperature; specifically, body temperatures were, on average, 2.4°C warmer and the preferred body temperature was 2.3°C higher. Meanwhile, lower critical limit increased by 1.2°C and the upper thermal limit increased

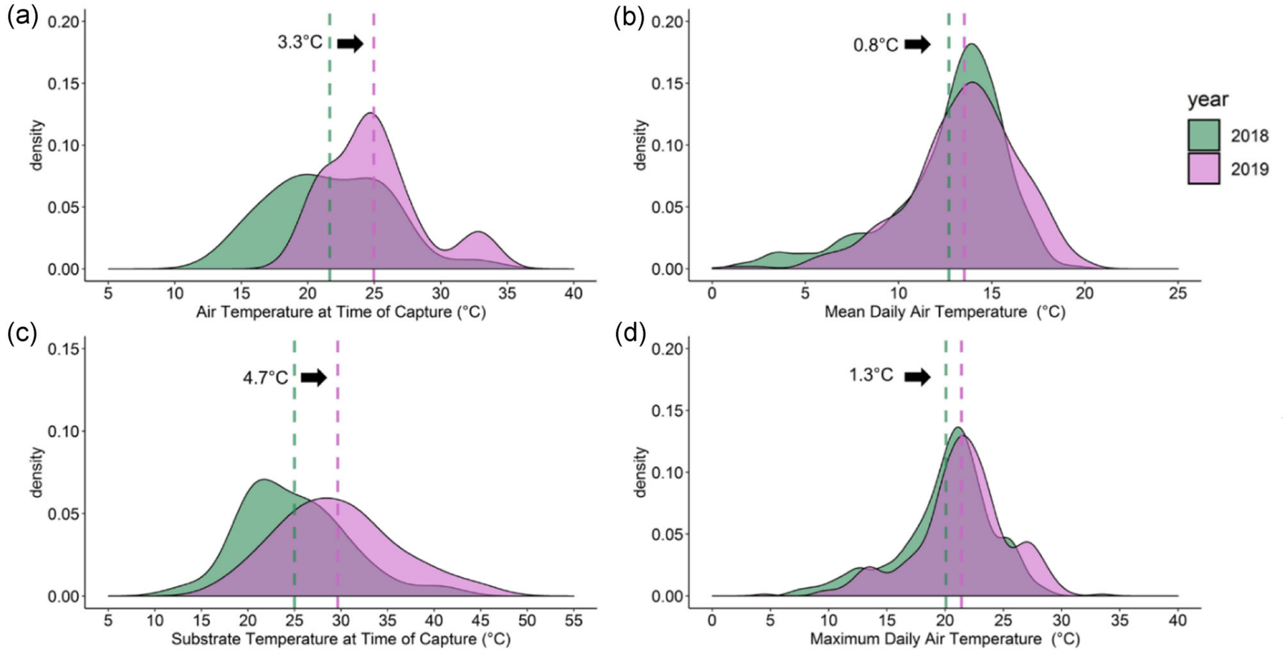

FIGURE 2 Distributions of macroclimatic (mean and maximum daily air temperatures gathered from nearby weather stations) and microclimatic conditions (air and substrate temperature at site of lizard capture) at the field site in Hidalgo, Mexico. (a) Air temperature at time of capture. (b) Mean daily air temperature. (c) Substrate temperature measured at the time of lizard capture and (d) Maximum daily air temperature. The thermal distributions for each variable are color coded by year, with measurements from 2018 given in green and measurements from 2019 shown in purple. The dashed lines (also color coded by year) denote the mean of each temperature distribution. The arrows denote the direction of temperature change from 2018 to 2019, and the magnitude of the corresponding change is also given [Color figure can be viewed at wileyonlinelibrary.com]

by 1°C from 2018 to 2019. There was no effect of age class, sex, or logSVL on any physiological traits (Table 2). Air temperature at time of capture (T_a) only had a significant impact on T_b (Table 2). Seven individuals (all adult males) collected in 2018 were recaptured in 2019. For this subset of resampled individuals, we found significant increases in T_{pref} (mean ± standard error [*SE*]: 2018 = 31.9 ± 0.5; 2019 = 36.1 ± 0.4) and CT_{min} (mean ± *SE*: 2018 = 4.0 ± 0.4; 2019 = 5.6 ± 0.6) between years, but not in T_b or CT_{max} (Table 3 and Figure 4). Although T_b and CT_{max} of individuals between years were not significantly different, the direction of the shift in mean values was positive.

4 | DISCUSSION

Across the globe 2019 was a record year for both average and maximum temperatures (NOAA, National Centers for Environmental Information, 2020), and our field site was no exception to this pattern. In comparison to 2018, central/northern Mexico was hotter in 2019 (Servicio Meteorológico Nacional, 2020). In the Mexican state of Hidalgo (where this study took place), the mean annual temperature was 18.7°C in 2018 and 19.8°C in 2019 (Servicio Meteorológico Nacional, 2020). The goal of our study was to examine whether and how thermal traits shifts across years in a natural population of a montane horned lizard, *P. orbiculare*, and consider how physiological shifts might shape their vulnerability to ongoing climate change. Our results clearly indicate that thermal traits respond rapidly to changes in the local environment. In 2019, four key aspects of thermal physiology—body temperature, the preferred temperature, the critical thermal minimum, and the critical thermal maximum—were all significantly higher than in 2018.

Increases in heat tolerance, cold tolerance, and the preferred temperature across years hint at potentially rapid evolutionary change because the period between observations (1 year) spans the generation time for this species (Suárez-Rodríguez et al., 2018), but this possibility requires further exploration. Due to the successful recapture of individuals between years, we were able to investigate whether intragenerational plasticity was contributed to increases in thermal physiology. Even with a small recapture success between years ($n = 7$), we detected clear increases in CT_{min} and T_{pref} that are consistent in magnitude with the whole-population trends that we observed. Thus, it is possible (if not more likely) that increases in these two traits reflect, at least in large part, phenotypic plasticity in response to warmer temperatures (Bonamour, Chevin, Charmantier, & Teplitsky, 2019). Our findings echo patterns found in previous studies, which indicate that CT_{min} and T_{pref} exhibit more within-generation plasticity than CT_{max} (Clusella-Trullas & Chown, 2014; Gvoždík, 2012; Kingsolver & Huey, 1998). Thus, the ability for organisms to dynamically alter some aspects of their physiology to fluctuating environments appears to be quite strong.

Despite a population-level increase in heat tolerance, we did not observe any intragenerational shifts in heat tolerance between 2018

TABLE 2 Mixed models evaluating year, logSVL, sex, age class (categorical), and air temperature at time of capture (T_a) on thermal traits of *Phrynosoma orbiculare* (see text for details)

Trait mean ± *SE* (*n*)	Effect	*F* value; *p* value
T_b	Year	**$F_{1, 48} = 5.20$; $p = .027$**
	logSVL	$F_{1, 48} = 0.01$; $p = .934$
2018 = 31.0 ± 0.96 (28)	Sex	$F_{1, 48} = 1.93$; $p = .171$
2019 = 33.4 ± 0.74 (26)	Age class	$F_{1, 48} = 0.17$; $p = .681$
	T_a	**$F_{1, 48} = 21.78$; $p < .001$**
T_{pref}	Year	**$F_{1, 48} = 35.60$; $p < .001$**
2018 = 32.6 ± 0.28 (28)	logSVL	$F_{1, 48} = 0.12$; $p = .731$
2019 = 34.9 ± 0.25 (27)	Sex	$F_{1, 48} = 0.02$; $p = .90$
	Age class	$F_{1, 48} = 0.19$; $p = .664$
	T_a	$F_{1, 48} = 0.08$; $p = .785$
CT_{min}	Year	**$F_{1, 42} = 14.64$; $p < .001$**
2018 = 4.38 ± 0.21 (23)	logSVL	$F_{1, 42} = 3.89$; $p = .055$
	Sex	$F_{1, 42} = 2.96$; $p = .092$
2019 = 5.62 ± 0.3 (26)	Age class	$F_{1, 42} = 1.72$; $p = .196$
	T_a	$F_{1, 42} = 2.68$; $p = .109$
CT_{max}	Year	**$F_{1, 42} = 7.54$; $p = .009$**
2018 = 37.78 ± 0.23 (23)	logSVL	$F_{1, 42} = 0.73$; $p = .396$
	Sex	$F_{1, 42} = 0.25$; $p = .620$
2019 = 38.79 ± 0.27 (26)	Age class	$F_{1, 42} = 1.44$; $p = .238$
	T_a	$F_{1, 42} = 0.99$; $p = .756$

Note: p-values < .05 are presented in bold font. Abbreviations: CT_{max}, critical thermal maximum; CT_{min}, critical thermal minimum; *SE*, standard error; SVL, snout-to-vent length; T_b, body temperature; T_{pref}, preferred body temperature.

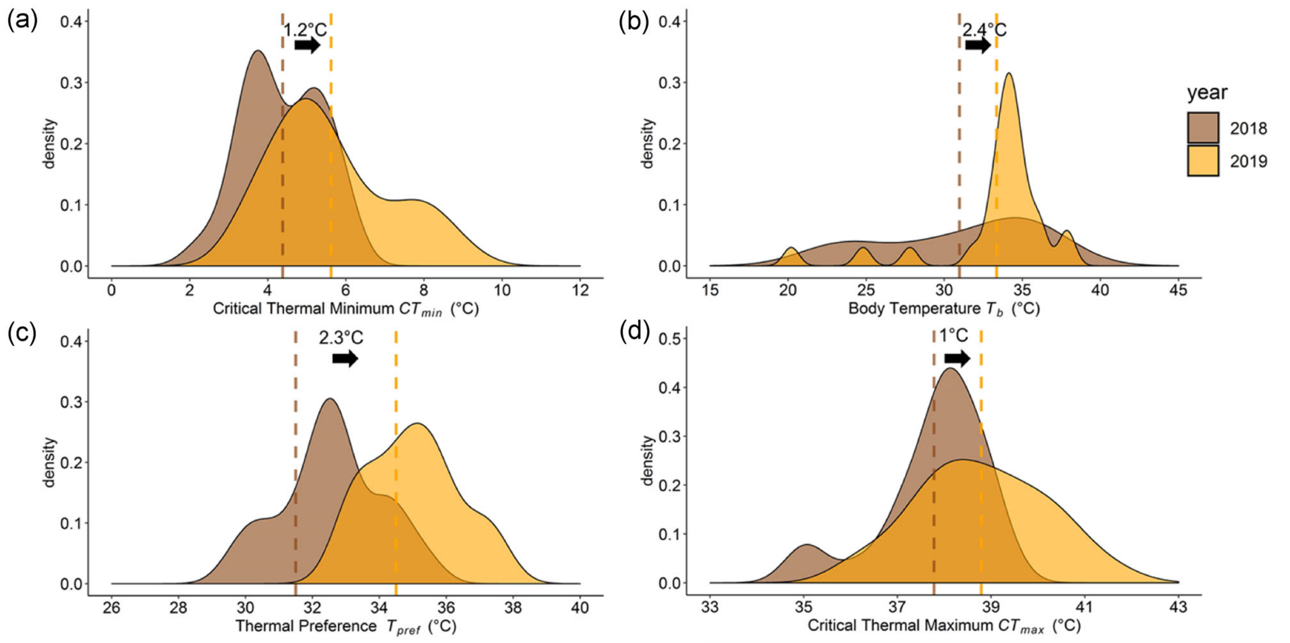

FIGURE 3 Distributions of (a) critical thermal minimum, (b) field measured body temperature, (c) thermal preference, and (d) critical thermal maximum. The thermal distributions for each variable are color coded by year, with measurements from 2018 given in brown and measurements from 2019 shown in orange. The dashed lines (also color coded by year) denote the mean of each temperature distribution. The arrows denote the direction of temperature change from 2018 to 2019, and the magnitude of the corresponding change is also given [Color figure can be viewed at wileyonlinelibrary.com]

and 2019. One possibility for this mismatch is that we lack the statistical power to detect a difference because CT_{max} increased only 1°C between years. Nonetheless, the population-level increase in CT_{min} was comparably modest (1.2°C), and an upward shift was also observed in resampled individuals. Another possibility (i.e., not necessarily mutually exclusive) is that heat tolerance exhibits a weaker long-term acclimatization response, meaning that intragenerational shifts in CT_{max} might not generally be expected on the timescale of our study. When confronted with a heat stress event (such as a heat wave), lizards will typically respond with a hardening response (a rapid, transient increase in heat tolerance) that arises over a short period of time (Maness & Hutchison, 1980), such as a 6–12 hr, and persists only for a few days (Gilbert & Miles, 2019; Phillips et al., 2016). In this study, we tested for intra-individual shifts in CT_{max} on the scale of approximately a year. Thus, there is the potential that, on shorter time scales between our sampling periods,

TABLE 3 Individual repeated measures of *Phrynosoma orbiculare* of body temperature (T_b), preferred body temperature (T_{pref}), critical thermal minimum (CT_{min}), and critical thermal maximum (CT_{max}) between 2018 and 2019

	T_b		T_{pref}		CT_{min}		CT_{max}	
ID	2018	2019	2018	2019	2018	2019	2018	2019
2	25.2	34.6	30.4	37.2	5.5	8.9	37.9	37.9
5	22.2	24.8	37.6	37.5	2.3	5.1	38.8	40.0
6	21.5	20.2	32.7	37.1	3.9	5.7	38.0	38.8
12	35.5	32.9	30.0	35.3	3.9	4.9	38.1	37.9
20	34.7	37.7	32.4	34.8	3.8	4.6	38.8	37.3
22	34.2	35.5	32.0	34.7	4.5	4.6	35.0	39.2
27	24.2	33.9	33.1	36.1	NA	5.2	NA	41.6
Mean ± *SE*	28.2 ± 2.4	31.4 ± 2.4	31.9 ± 0.5	36.1 ± 0.4	4.0 ± 0.4	5.6 ± 0.6	37.8 ± 0.6	39.0 ± 0.6
Statistics	$t = -1.738$; $df = 6$; $p = .130$		**$t = -6.953$; $df = 6$; $p = .020$**		**$t = -3.204$; $df = 5$; $p = .020$**		$t = -0.952$; $df = 5$; $p = .385$	

Note: Results highlight the within-individual variation of thermal traits between years. *p*-values < .05 are presented in bold font.
Abbreviations: *df*, degrees of freedom; *SE*, standard error.

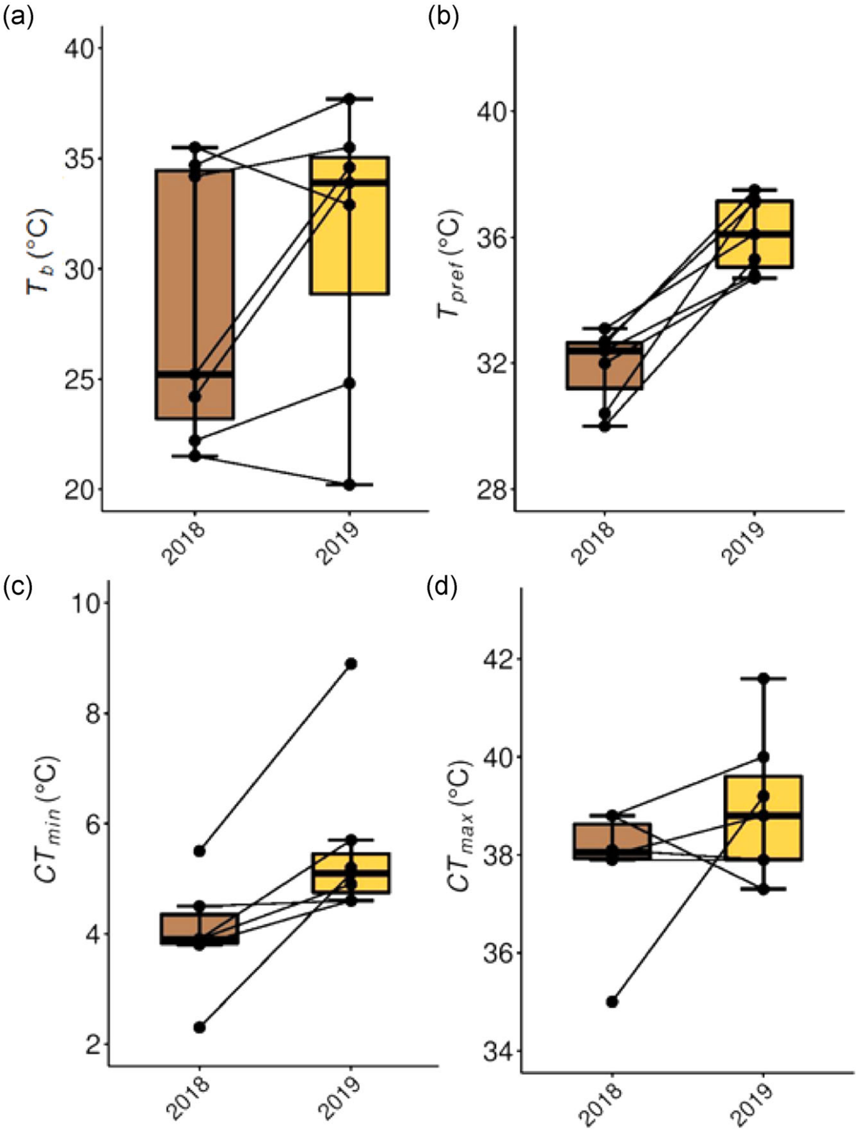

FIGURE 4 Boxplot comparisons of thermal physiological traits of individual shifts between years. (a) Field measured body temperature, (b) preferred body temperature, (c) critical thermal minimum, and (d) critical thermal maximum for individuals measured in 2018 and 2019. Boxes are color coded by year with 2018 in brown and 2019 in orange. Lines connect the same individual measured in both 2018 and 2019. The dark bar represents the median, the boxes are the interquartile range, and the whiskers roughly provide the 95% confidence interval for comparing medians [Color figure can be viewed at wileyonlinelibrary.com]

individual organisms responded plastically to heat stressors, and that we missed these shifts due to the long temporal gap between our measurements. Given that we observed an increase in CT_{max} at the population level without a concomitant increase at the individual level, however, it is further possible that greater heat tolerance in 2019 represents an evolved (intergenerational) response. For example, increasing temperatures may have resulted in nonrandom survivorship and/or differential reproductive success in this population of *P. orbiculare*. Previous studies have documented that climate-induced selection on lizard physiology can be strong, often resulting in rapid phenotypic shifts over short time periods (e.g., Campbell-Staton et al., 2017; Gilbert & Miles, 2019; Leal & Gunderson, 2012; Logan, Cox, & Calsbeek, 2014), but macroevolutionary studies suggest that climatic adaptation, particularly in upper physiological limits, can be surprisingly sluggish (Bodensteiner et al., 2020; Farallo, Muñoz, Uyeda, & Miles, 2020; Salazar, Castañeda, Londoño, & Muñoz, 2019). Another possibility is that the higher heat tolerance observed in 2019 reflects warmer maternal incubation conditions; warmer embryonic conditions in reptiles can induce overexpression of heat shock proteins that might be retained into adulthood (Gao et al., 2014). In the velvet gecko (*Amalosia lesueurii*), however, warmer embryonic conditions lead to a lower heat tolerance in hatchlings; it is unknown if these patterns persist into adulthood (Dayananda, Murray, & Webb, 2017). Another study found no effect of embryonic conditions on heat tolerance (Llewelyn et al., 2018). Thus, further work is necessary to discover the mechanistic basis for the heat tolerance shift.

On sampling days in 2019 air temperatures were 3.3°C warmer and substrate temperatures were 4.7°C warmer than on sampling days in 2018. As such, we expected a higher body temperature in field-active lizards in 2019 relative to 2018. One intriguing outcome from our study is that body temperature increased between years at the level of the whole population, but not among resampled individuals. There may be several reasons for this pattern. In 2019, the resampled lizards were larger than the rest of the population (two-way analysis of variance: $F_{1,25} = 6.21$; $p = .02$). If larger, older lizards are able to outcompete smaller lizards for access to cooler microsites (e.g., Melville, 2002), then this might explain why resampled individuals exhibited a lower body temperature. This idea hinges on active thermoregulation by *P. orbiculare*, for which some evidence exists (Pardo-Ramirez, unpublished data), but nonetheless requires a deeper consideration using an explicit hypothesis testing framework (sensu, Hertz et al., 1993). Another possibility is that we lack the statistical power to detect a difference in body temperature among resampled individuals. Body temperature is a noisy variable: our range of observations is 17.8°C, which is more than twice as high as for T_{pref} (range = 7.9°C) and almost three times higher than for CT_{min} and CT_{max} (range = 6.6°C for both traits). With only seven resampled individuals, we suspect that we simply lacked the statistical resolution to detect differences (if they do exist). Moreover, body temperature can fluctuate substantially between days and across seasons, limiting our ability to draw robust conclusions. Despite extensive field effort, densities of *P. orbiculare* were low, which makes robust resampling of individuals (particularly for noise-prone variables) exceptionally challenging.

As global temperatures continue to rapidly rise, a question that looms large is whether organisms, especially ectotherms, will be able to keep pace with environmental change (Moritz & Agudo, 2013; Quintero & Wiens, 2013; Sinervo et al., 2010). Our general finding is that thermal traits can and do readily respond to increasing temperatures. These results are promising, as they suggest that lizards, even geographically restricted montane species like *P. orbiculare*, are equipped to tackle rapid and dramatic thermal increases in their habitat. Nonetheless, while shifts in thermal traits may confer resilience in the short term, they may not be sufficient in the long term unless warming slows or reverses (Buckley, Ehrenberger, & Angilletta, 2015; Muñoz & Moritz, 2016; Oostra, Saastamoinen, Zwaa, & Wheat, 2018; Refsnider et al., 2018). Research suggests that physiological shifts may have hard limits beyond which phenotypes become inaccessible: In the rainforest skink *Lampropholis coggeri*, for example, whether through adaptive evolution or phenotypic plasticity, the hard upper limit for locomotor performance resides near 43°C (Muñoz et al., 2016; Phillips et al., 2016). Likewise, behavioral refuges from the heat might become scarcer as environments continue to warm (Kearney et al., 2009, Kearney, 2013; Sears et al., 2016). Therefore, as organisms are confronted with progressively more hostile thermal environments, activity periods are likely to constrict, especially once phenotypic limits are reached and the costs of thermoregulation mount. If such activity constriction becomes severe, populations may decline in size or even go extinct (Basson, Levy, Angilletta, & Clusella-Trullas, 2017; Kearney, 2013; Sears et al., 2016; Sunday et al., 2014). While it is clear that plasticity can at least partially restructure the thermal phenotypes of a wild population of *P. orbiculare*, we know far less of the intrinsic limits to this ability. Moving forward, directly incorporating estimates of physiological acclimatization into dynamic energy budgets (sensu, Riddell, Odom, Damm, & Sears, 2018) will help improve our predictions of species distribution modeling in a changing world.

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DATA AVAILABILITY STATEMENT

Data will be made available in the data repository Dryad upon manuscript acceptance.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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CAPÍTULO 2. EVOLUCIÓN FISIOLÓGICA Y DE HISTORIAS DE VIDA EN UNA FAMILIA DE LAGARTIJAS

2.1. *Exceptional parallelism characterize the evolutionary transition to live birth in phrynosomatid lizards*

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 rates[8] 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 reproduction[10] 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	Body mass is similar among oviparous and viviparous species, but body temperature is lower in viviparous species.
ii	Body mass is higher and body temperature is lower in viviparous species.
iii	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
iv	Offspring size is similar between oviparous and viviparous species, but annual fecundity is lower in viviparous species.
v	Offspring size and annual fecundity are lower in viviparous species.
vi	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 vertebrates[6]. Here, we leveraged the prolific evolution of viviparity in phrynosomatid lizards, a lineage renowned for repeated transitions to live birth[13], 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 allotment[16]. 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 continuum[17]. 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**).

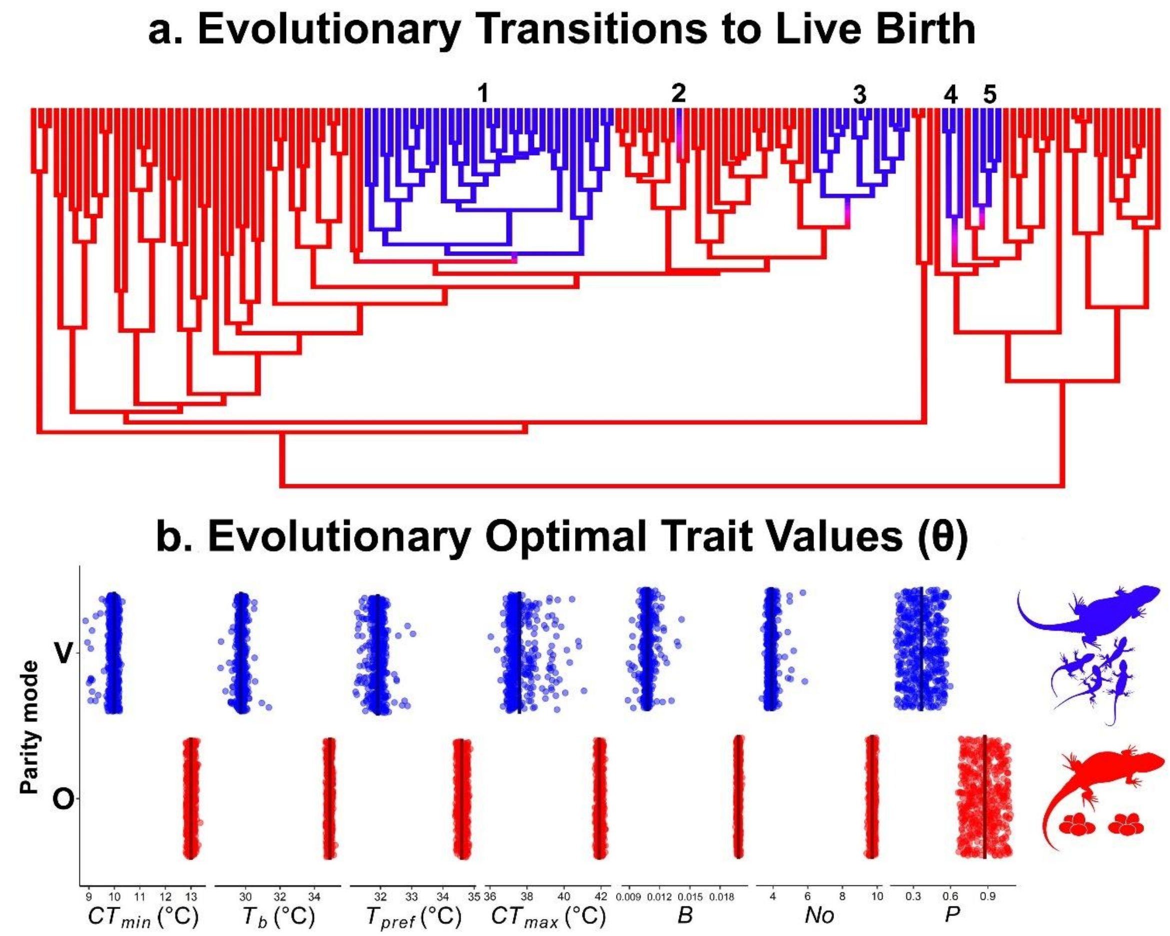

Fig. 1 Parallel reductions in physiological and life history traits are associated with viviparity in phrynosomatid lizards. **a** Five evolutionary transitions from egg-laying (red) to live-bearing (blue) occurred in phrynosomatids. **b** Viviparous lineages are characterized by reductions in the evolutionary 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 ($\log_{10}$body mass= 0.288$\log_{10}$SVL + 1.522, *p*=0; Supplementary Table 4), was positively correlated with offspring size ($\log_{10}$offspring size= 0.289$\log_{10}$SVL + 0.906, *p*<0.001; **Fig. 2a**; Supplementary Table 4) and litter/clutch size ($\log_{10}$ litter or clutch size =1.132$\log_{10}$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 females[26] (θ= 61.1 mm SVL; Supplementary Table 2) remains unchanged because any size shift also affects the quality and quantity of offspring.

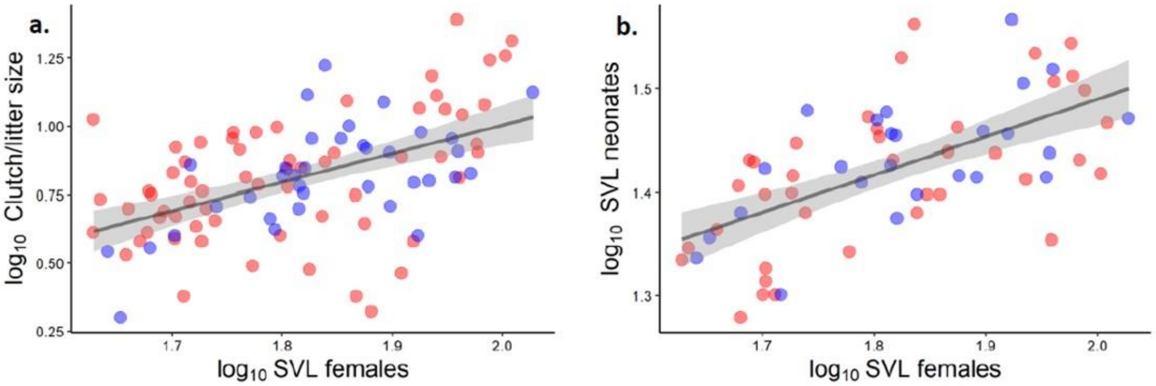

Fig. 2 Influence of dam's body size on clutch/litter size (**a**; y= 1.1321x -1.2362, *p*<0.01) and on the body size of neonates (**b**; y= 0.2893x + 0.9064, *p*<0.01) in phrynosomatid lizards. Blue circles correspond to viviparous species whereas red circles correspond to oviparous species. 95% confidence 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 (θ= 29.7°C) when compared to their egg-laying counterparts (θ= 34.9°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 species[27]. 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 nests[31] tends to be lower than the optimal body temperature of oviparous adults (θ= 34.9°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 habitats[13] 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 (θ= 9.95 °C) than the oviparous species (θ= 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 environments[13]. 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 labile[32] and often correlates tightly with the minimum temperatures ectotherms experience in their environments[33]. This tight connection often reflects limited opportunities to behaviorally buffer against the cold, which results in rapid cold adaptation to prevailing conditions[34].

We also detected parallel evolutionary reductions in the phenotypic optimum for heat tolerance (θ= 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 (θ= 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±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.

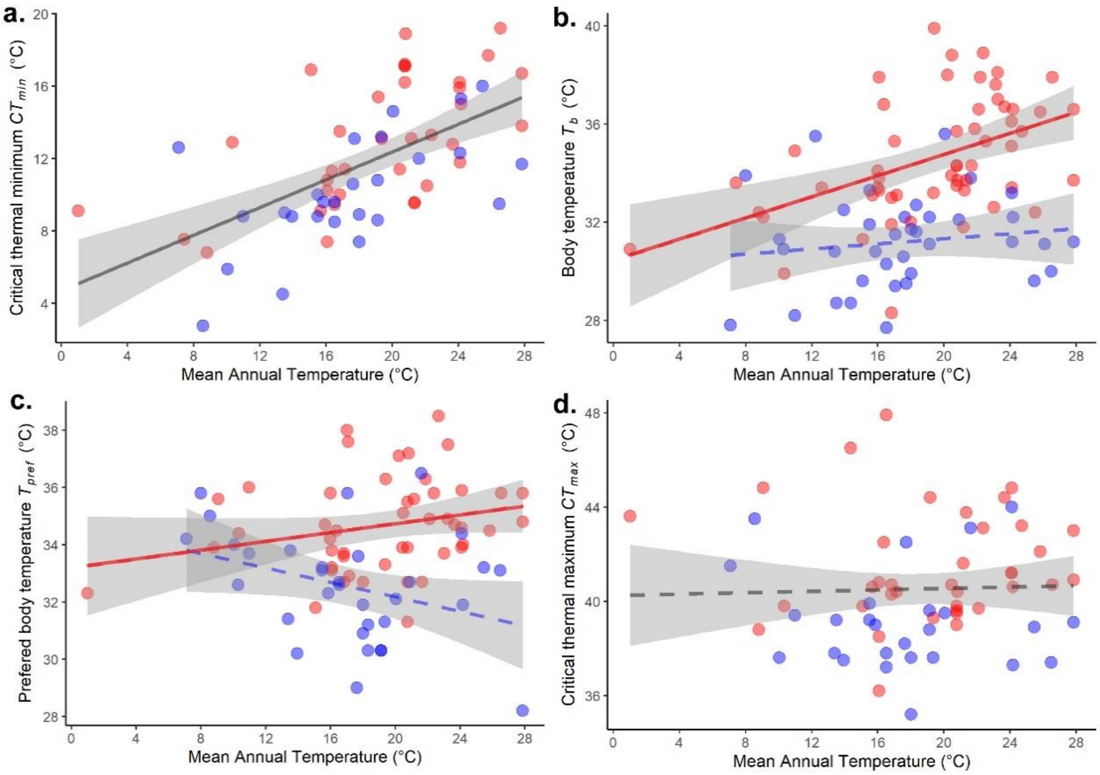

Fig. 3 Influence of mean annual temperature (MAT) on cold tolerance (**a**; y=0.354x + 5.639, *p*=0;), field-body temperature (**b**; for oviparous: y=0.230x + 30.3, *p*=0, and for viviparous: y=0.055x + 30.69, *p*=0.4), preferred body temperature (**c**; for oviparous: y=0.094x + 32.78, *p*=0.03, and 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 ago[35], 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 (θ= 0.83 g; Supplementary Table 2) and offspring size (θ= 26.4 mm snout-vent-length; Supplementary Table 2) do not vary between viviparous and oviparous species. The retention of optimal offspring size[36] 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 year[39]. 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 temperatures[22], 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.7[42] and concatenation and manual refinement using Mesquite ver. 3.6[43]; 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* specimen[45]). 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 2.5.2[47] and run on CIPRES[48]. 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 1.7[49]. 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.1.8.4[49]. 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 assignations 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, Mexico[52]. 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 ectotherms[53] 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 lizards[33], the preferred body temperature (T_{pref}) in a laboratory thermal gradient[54], 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 offspring[28]. 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 gravis/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 females[29], 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 thermoregulation[56]. 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 species[33]. Into each pipe model, we inserted one temperature data logger (Thermochron iButton; model DS1921G), which recorded temperature (±0.1°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 counterparts[27]. 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 habitat[54]. We estimated E for each species following the equation proposed by Hertz et al.[54]:

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

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. $\overline{de}$ 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 reproduction[39]. Second, oviparous species are able to produce eggs in multiple clutches per year[61], whereas viviparous species are typically able to produce only one litter in the same unit time[39]. 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 modes[36]. 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]:

$$I= i_0M^{3/4}\, e^{-E/kT}$$

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 that 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= $10^{\text{log10body mass}}$). 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 mass[9]. 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 mapping[68] 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 package[69]. We selected the ER model of character evolution because was it the best-supported model (ΔAICC=0, weight= 0.65) in comparison to a symmetrical model (SYM; ΔAICC=2.1, weight= 0.22) and with an all-rates-different model (ARD; ΔAICC=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 package[69].

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 package[69].

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 OUwie[70] and the 500 stochastic character maps of parity mode built with the *make.simmap* function in the R package phytools[69]. 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 (AIC_C) that incorporates a correction for small size[71]. 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 nlme[72].

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 package[69]. The threshold model is used to test for evolutionary covariation between continuous and discrete traits[74]. 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 x 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 elevation[13]) 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 SLOUCH[75].

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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.

DISCUSIÓN GENERAL

En el presente siglo, el cambio climático (CC) podría ocasionar la extinción de al menos una de cada cinco especies en algunos grupos de animales ectotermos[2,3], a menos que la selección natural favorezca variables conductuales y fisiológicas que permitan a las poblaciones persistir al nuevo ambiente térmico[13,17,19]. La capacidad de mantener la temperatura corporal (T_b) dentro del intervalo de temperatura corporal preferida (T_{pref}) mediante termorregulación conductual y la plasticidad de la fisiología térmica de la T_{pref}, de la tolerancia al frío (CT_{min}) y de la tolerancia al calor (CT_{max}) podría permitir a los animales ectotermos sobrevivir y reproducirse en un nuevo ambiente térmico asociado con CC[6,26,27]. En la presente tesis se eligió como modelo de estudio a las lagartijas de la familia Phrynosomatidae, una familia distribuida ampliamente en Norte América, para poner a prueba la adaptación conductual y fisiológica de un grupo de animales ectotermos a su ambiente térmico. La tesis estuvo dividida en dos capítulos, en el primero se documentó la estrategia de termorregulación conductual y la plasticidad de la fisiología térmica en dos poblaciones de lagartijas (*Sceloporus torquatus* y *Phrynosoma orbiculare*) y en el segundo se documentó la evolución conductual y de la fisiológica térmica en más de cien especies de Phrynosomatidos mediante el método comparativo filogenético.

Los resultados mostraron que la conducta de termorregulación de las lagartijas de la familia Phrynosomatidae es altamente efectiva y permite a los individuos mantener su temperatura corporal dentro de su intervalo preferido a pesar de la variación térmica diaria o estacional. Por ejemplo, en el Jardín Botánico de la UNAM, sitio de estudio para *S. torquatus*, la temperatura del sustrato puede llegar hasta los 60°C y la temperatura ambiental promedio varía de 11.2°C en invierno a 23.5°C en primavera. A pesar de la variación térmica amplia, los individuos de *S. torquatus* mantienen mediante termorregulación conductual su temperatura corporal dentro de un intervalo estrecho (31.1-34.9°C). La alta efectividad para mantener la temperatura corporal dentro de un intervalo preferido mediante ajustes conductuales es típica de las lagartijas de la familia Phrynosomatidae[14,28,29,30,31,32,33,34] y se comprobó para las 64 especies de las que se obtuvieron y analizaron datos de termorregulación conductual. El mantenimiento de la T_b dentro del intervalo de T_{pref} mediante termorregulación conductual apoyaría la idea de que los phrynosomatidos podrán amortiguar las temperaturas ambientales altas ocasionadas por CC. Sin embargo, conforme los días se vuelven más calurosos, las lagartijas están reduciendo su horario de actividad (evitando temperaturas extremas)[10], lo cual ocasionará que los individuos no tengan tiempo suficiente para alimentarse y reproducirse y que las poblaciones decrezcan hasta su extinción[3,33,35,36]. Entonces, la termorregulación conductual es útil para que los individuos evadan temperaturas letales, pero no será suficiente para que las poblaciones de phrynosomatidos persistan ante el escenario actual de CC, a menos que esté acompañada por cambios plásticos en los límites de tolerancia térmica y/o en la T_{pref}.

Si los individuos cambian su CT_{min}, CT_{max} y T_{pref} de forma correlacionada con la temperatura ambiental, es posible que puedan sobrevivir y mantener su actividad en ambientes térmicos extremos[13,19,37]. Nuestros resultados indican que la CT_{min} responde de forma rápida y plástica al ambiente térmico, lo cual es común en lagartijas[27,38,39]. Por ejemplo, la CT_{min} de *Sceloporus torquatus* es de 10.4°C en primavera (temperatura ambiental de 19°C), pero llega hasta 6.4°C en invierno (temperatura ambiental de 14.3°C). Al realizar modelos filogenéticos de mínimos cuadrados generalizados (PGLS), se encontró que la plasticidad fisiológica en CT_{min} se ve reflejada en patrones adaptativos; a menor temperatura ambiental, mayor tolerancia al frío. El intervalo interespecífico de la CT_{min} es amplio, por ejemplo, la CT_{min} de *Phrynosoma hernandesi* es de 2.75°C[40] y de *Sceloporus chrysostictus* es de 19.2°C. La evolución rápida y el intervalo amplio de la CT_{min} suele estar asociado con ausencia de termorregulación conductual nocturna en especies de lagartijas diurnas[39]. En sitios donde la temperatura nocturna cambia de forma amplia entre meses o estaciones, los individuos están expuestos a una variación amplia de su temperatura corporal durante la noche[41]. Esa variación térmica es una presión de selección continua; individuos con mayor tolerancia al frío (o mayor plasticidad en su CT_{min}) tienen mayor supervivencia y éxito reproductor[27,39, 41]. Aunque la CT_{min} evoluciona de forma rápida[39,41], esa respuesta adaptativa será importante solo en sitios en los que la temperatura ambiental decrezca, ya que la tendencia actual de CC es el calentamiento global[1,42], entonces resulta más importante la evolución de la CT_{max} que de la CT_{min}.

Los estudios poblacionales mostraron que la media poblacional de la CT_{max} de *S. torquatus* no cambia de forma correlacionada con la temperatura ambiental y mediante marcaje-recaptura, se documentó que la CT_{max} de *P. orbiculare* no cambia a través de su vida, a pesar de que su ambiente térmico se está volviendo más cálido. La plasticidad nula de la CT_{max} también se vio reflejada en comparaciones interespecíficas. El análisis PGLS mostró que la CT_{max} de las lagartijas de la familia Phrynosomatidae no es una adaptación a la temperatura ambiental. En ambientes fríos o cálidos, las lagartijas no lograran tolerar temperaturas corporales mayores a las actuales. La adaptación nula de la CT_{max} a la temperatura ambiental es un patrón común en lagartijas y en otros animales ectotermos[27,39,43]. Los phrynosomatidos y otros ectotermos no pueden evolucionar a CT_{max} mayores debido a restricciones bioquímicas, por ejemplo, temperaturas corporales altas ocasionan desnaturalización de enzimas y efectos deletéreos a nivel celular, de sistemas y de organismo[5]. Entonces, evolucionar a tolerar temperaturas corporales mayores no será un mecanismo de respuesta de los phrynosomatidos al calentamiento global.

Con respecto a la evolución de la T_{pref}, los resultados indican que es una adaptación al ambiente térmico, pero solo en especies ovíparas. El análisis de PGLS mostró que las especies ovíparas prefieren temperaturas mayores a mayor temperatura ambiental, lo cual les permite mantener su desempeño y adecuación en sitios cálidos. Este ajuste ocasiona que las especies ovíparas de sitios cálidos mantengan temperaturas corporales altas y cercanas a su CT_{max} y que dependan de su conducta de termorregulación para no morir por sobrecalentamiento[26]. En promedio, las especies ovíparas de Phrynosomatidae prefieren temperaturas mayores que las especies vivíparas (34.6°C vs 31.9°C, respectivamente). Ya que las preferencias térmicas de las especies ovíparas no pueden exceder su CT_{max} (el cuál no evoluciona en respuesta a la temperatura ambiental) y que la T_{pref} de las especies vivíparas es baja, se sugiere que la evolución de la T_{pref} tampoco es una respuesta posible de los phrynosomatidos al CC. El patrón evolutivo de preferencias por temperaturas corporales altas en especies ovíparas, pero preferencias por temperaturas corporales bajas en especies vivíparas había sido documentado previamente para phrynosomatidos y otros escamados[3,44] y podría ser producto de un ajuste fisiológico asociado con la evolución de la viviparidad[45].

La temperatura corporal durante la incubación o gestación influye en el fenotipo de las crías[46]. Por ejemplo, se ha observado que, a temperaturas corporales altas, las crías de *Sceloporus jarrovii* nacen pequeñas, con deformidades, o muertas[47]. El efecto de la temperatura corporal de las especies vivíparas en el fenotipo de sus crías podría estar relacionado con aspectos metabólicos. La tasa metabólica es menor en especies vivíparas que en especies ovíparas[45,48]. Se encontró que la forma en que las especies vivíparas reducen su tasa metabólica, es manteniendo temperaturas corporales bajas mediante termorregulación conductual, sin cambios en la masa corporal. La preferencia por temperaturas corporales bajas es incluso más marcada durante la gestación[49,50]. Entonces, se considera que los ajustes en temperatura corporal, en lugar de ajustes en masa corporal, ocurren porque el tamaño de las hembras adultas influye directamente en el número y tamaño de su descendiencia[51]. Previamente, se había documentado que a menor tasa metabólica los individuos destinan menor energía a producción reproductiva[25]. Al incluir datos de fecundidad anual y tamaño de las crías, se encontró que, de forma paralela, las especies vivíparas también tienen una menor fecundidad anual que las especies ovíparas, pero en ambos modos de paridad se mantiene el mismo tamaño promedio de las crías[51]. Se sugiere que la menor fecundidad anual de las especies vivíparas, es consecuencia de una gestación prolongada[52] y se ve favorecida en sitios de baja depredación, como son los sitios de alta elevación[53,54,55], donde las especies vivíparas de phrynosomatidos son predominantes[20,56]. Sin embargo, el mismo tamaño corporal de crías para ambos modos de paridad está moldeado por selección natural. Se estimó que el óptimo evolutivo del tamaño las crías de especies ovíparas y vivíparas es de 26.4 mm (longitud hocico-cloaca). Es posible que ese tamaño de las crías se mantenga porque, en relación con el tamaño de su madre, crías extremadamente pequeñas o grandes, tengan menor supervivencia[57,58]. Finalmente, unir aspectos de fisiología térmica con teoría metabólica y de historias de vida permitieron conocer de mejor forma las respuestas y restricciones de los phrynosomatidos ante el CC.

En conclusión, la termorregulación conductual permite a las lagartijas de la familia Phrynosomatidae evadir temperaturas letales, pero no les permitirá responder al CC si no tienen tiempo suficiente para alimentarse y reproducirse. La adaptación de la CT_{min} a la temperatura ambiental permitirá a los phrynosomatidos tolerar temperaturas menores en los sitios en que la temperatura ambiental decrezca por CC. Sin embargo, la nula adaptación de la T_{pref} (en especies vivíparas) y de la CT_{max} (en especies ovíparas y vivíparas) a la temperatura ambiental, vuelve a las lagartijas de la familia Phrynosomatidae especialmente vulnerables al CC. Es posible que las proyecciones de riesgo de extinción para lagartijas en el presente siglo se cumplan, por lo que es urgente proponer y/o modificar las estrategias actuales de conservación y de mitigación del CC. La heterogeneidad térmica es clave para que los phrynosomatidos y otros animales ectotermos terrestres regulen conductualmente su temperatura corporal y mantengan un horario de actividad que les permita desarrollar todas sus actividades biológicas. Dicha heterogeneidad térmica ocurre principalmente, cuando existen parches naturales de sol y sombra en el ambiente, entonces, frenar las tasas actuales de deforestación y restaurar la cobertura vegetal en sitios perturbados podría ser la única alternativa (al menos a corto plazo) que permita a los phrynosomatidos persistir ante al CC.

CONCLUSIONES GENERALES

1. Las lagartijas de la familia Phrynosomatidae mantienen su temperatura corporal (T_b) dentro de su intervalo de temperatura corporal preferida (T_{pref}) mediante termorregulación conductual.
2. La temperatura corporal preferida (T_{pref}) de las especies de la familia Phrynosomatidae es una adaptación a la temperatura ambiental en especies ovíparas, pero no es una adaptación a la temperatura ambiental en especies vivíparas.
3. La tolerancia al frío (CT_{min}) de las especies de la familia Phrynosomatidae es una adaptación a la temperatura ambiental, pero la tolerancia al calor (CT_{max}) de las especies de la familia Phrynosomatidae no es una adaptación a la temperatura ambiental.

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