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Climate aridity and habitat drive geographical variation in morphology and thermo-hydroregulation strategies of a widespread lizard species

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Thermo-hydroregulation strategies involve concurrent changes in functional traits related to energy, water balance and thermoregulation and play a key role in determining life-history traits and population demography of terrestrial ectotherms. Local thermal and hydric conditions should be important drivers of the geographical variation of thermo-hydroregulation strategies, but we lack studies that examine these changes across climatic gradients in different habitat types. Here, we investigated intraspecific variation of morphology and thermo-hydroregulation traits in the widespread European common lizard (*Zootoca vivipara louislantzi*) across a multidimensional environmental gradient involving independent variation in air temperature and rainfall and differences in habitat features (access to free-standing water and forest cover). We sampled adult males for morphology, resting metabolic rate, total and cutaneous evaporative water loss and thermal preferences in 15 populations from the rear to the leading edge of the distribution across an elevational gradient ranging from sea level to 1750 m. Besides a decrease in adult body size with increasing environmental temperatures, we found little effect of thermal conditions on thermo-hydroregulation strategies. In particular, relict lowland populations from the warm rear edge showed no specific ecophysiological adaptations. Instead, body mass, body condition and resting metabolic rate were positively associated with a rainfall gradient, while forest cover and water access in the habitat throughout the season also influenced cutaneous evaporative water loss. Our study emphasizes the importance of rainfall and habitat features rather than thermal conditions for geographical variation in lizard morphology and physiology.

ADDITIONAL KEYWORDS: aridity – evaporative water loss – lizards – metabolism – morphology – reptiles – thermal preferences.

INTRODUCTION

Ongoing climate change dramatically impacts biodiversity, and increasing our ability to predict future ecological effects of climate changes is one of the main challenges facing research in ecology today (Bellard *et al.*, 2012). In widely distributed organisms, gradient analyses of intraspecific variation along contrasted climate conditions can inform about the environmental sensitivity and capacity of different species to adapt to future changes (Blois *et al.*, 2013). Terrestrial ectotherms are sensitive to environmental

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*Corresponding author. E-mail: chloe.chabaud@normalesup.org temperatures and highly vulnerable to global warming, especially to heat and drought stress, which impairs activity, causes physiological stress and can reduce fitness and population growth (Huey *et al.*, 2009, 2012; Sinervo *et al.*, 2010; Kubisch *et al.*, 2016).

Terrestrial ectotherms can cope with heat and drought risks by changes in thermoregulation (i.e. heat exchange and body temperature regulation) and hydroregulation traits (i.e. water balance regulation), collectively referred to as their 'thermo-hydroregulation' strategies (Rozen-Rechels *et al.*, 2019). Nonetheless, large uncertainties remain about the respective roles of physiological and behavioural responses and the geographical variability of body temperature and water balance regulation. Behavioural and physiological maintenance of an optimal body temperature and hydration state depends to a large extent on spatiotemporal variability in operative temperatures, water availability and water vapour deficit (Rozen-Rechels *et al.*, 2019; Sears *et al.*, 2019).

Historically, however, research in comparative physiology has focused on the thermal biology of terrestrial ectotherms (Huey *et al.*, 2012; Sunday *et al.*, 2014; Seebacher *et al.*, 2015; Artacho *et al.*, 2017; Caldwell *et al.*, 2017; Rutschmann *et al.*, 2020). For example, populations of the same species of lizards can display different critical thermal limits depending on local thermal conditions, particularly those characterizing the cold end of the thermal performance curves (Pontes-da-Silva *et al.*, 2018; Herrando-Pérez *et al.*, 2020; Bodensteiner *et al.*, 2021). Another general finding concerns the metabolic cold adaptation in energy expenditure, whereby organisms from cooler climates have higher basal metabolic rates, probably to compensate, in part, for the slow development and pace of life associated with low environmental temperatures in ectotherms (Seebacher, 2005; Dupoué *et al.*, 2017a; Pettersen, 2020). Instead, intraspecific studies on thermal preferences (i.e. preferred body temperatures in the absence of thermoregulation costs) have led to inconclusive results, with some authors demonstrating geographical differences in thermal preferences (Trochet *et al.*, 2018; Rozen-Rechels *et al.*, 2021) whereas others suggest that thermal preferences are more evolutionarily rigid than other thermal biology traits (Clusella-Trullas & Chown, 2014). In addition, we lack knowledge on how intraspecific variations correspond to the observed variation between species.

To date, most studies of geographical variation in reptile thermal traits have focused on thermal clines, such as broad elevational or latitudinal gradients in air temperatures (Gvoždík & Castilla, 2001; Osojnik *et al.*, 2013; Zamora-Camacho *et al.*, 2013; Clusella-Trullas & Chown, 2014; Trochet *et al.*, 2018; Plasman *et al.*, 2020). Such broad thermal clines usually confound with variation in precipitation, making the two factors difficult to distinguish. Nonetheless, habitat characteristics can amplify or buffer thermal variability, and recent studies have highlighted the importance of water availability and microclimatic conditions when studying responses of terrestrial ectotherms to environmental modifications (Miller & Lutterschmidt, 2014; Wegener *et al.*, 2014; Chiacchio *et al.*, 2020). In squamate reptiles, availability of water and suitable microhabitats are essential for optimal thermoregulation and hydroregulation, because behavioural exploitation of microhabitats (such as wet shelters from the vegetation) can buffer these species from the deleterious effects of a temperature increase or a rainfall reduction (Sears *et al.*, 2016; Rozen-Rechels *et al.*, 2020). In addition, in heliothermic species the costs of maintaining high body temperature usually increase when those species face a reduction of water availability in the environment, leading to the selection of a suboptimal body temperature (Rozen-Rechels *et al.*, 2020). Variation in morphological traits can also be affected strongly by water availability and microhabitat features (Ashton, 2001; Olalla-Tárraga *et al.*, 2009, Roitberg *et al.*, 2020). Teasing apart the effects of thermal gradients, water availability and local habitats on thermo-hydroregulation strategies and morphology requires comparative analyses along independent clines of ambient temperature and rainfall in sites with contrasted microhabitat features.

Another limitation is that comparative studies of ectotherm hydroregulation strategies still lag behind those of thermoregulation (Pirtle *et al.*, 2019). Hydroregulation involves both behavioural and physiological mechanisms, such as behavioural activity and shelter use, behavioural microhabitat selection or physiological mechanisms of evaporative water loss through the skin (Pirtle *et al.*, 2019). Skin resistance to evaporative water loss in lizards and snakes is determined primarily by the presence of a lipid layer in the epidermis, which acts as a physical barrier to water loss and determines total evaporative water loss (TEWL) rates (Roberts & Lillywhite, 1980). Plastic changes in TEWL rates over a few weeks or months have been demonstrated in some lizards and snakes when individuals are exposed to different air moisture or water availability conditions (Kobayashi *et al.*, 1983; Moen *et al.*, 2005). Phylogenetic analyses of TEWL in lacertid lizards also indicate that this trait is more evolutionarily labile and more variable than thermal preferences and that it evolves towards lower values in warmer environments (Garcia-Porta *et al.*, 2019). However, studies of covariation in TEWL and thermoregulation traits are rare, and they suggest that the two sets of functional traits might often vary independently from each other (Sannolo *et al.*, 2020; S'khifa *et al.*, 2020).

Here, we performed a comparative study in the lacertid *Zootoca vivipara louislantzi* Arribas, 2009, which is a ground-dwelling lizard with an oviparous reproductive mode. We examined variation in thermoregulation and hydroregulation traits across a geographical gradient in south-western France, from relict populations located at the hot distribution margin to populations located at the colonization front in cold, highland habitats (Dupoué *et al.*, 2021). Using an integrative approach, we quantified geographical variation in the following: (1) thermal preference (T_{pref}); (2) hydroregulation physiology (TEWL and its cutaneous subcomponent, CEWL); (3) energy metabolism (resting metabolic rate); and (4) morphology (body size, body surface, body condition and caudal reserve) of adult male lizards from 15 populations. We also evaluated physiological dehydration through osmolality to investigate its correlation with climatic conditions and habitat. To avoid confounding effects of inter-individual differences attributable to age, sex or seasonality, we sampled only sexually mature males during the reproductive season. We also compared our studied populations with those of two taxonomic outgroups sampled with the same technique, including two nearby French populations of the closely related subspecies *Zootoca vivipara vivipara* with a viviparous reproductive mode and one population of the distantly related wall lizard, *Podarcis muralis*, from semi-mesic, saxicolous environments (Garcia-Porta *et al.*, 2019). This sampling design allowed us to test whether hydroregulation physiology and energy metabolism traits are more flexible than thermal preferences, whether those traits covary or vary independently from each other, and whether intraspecific variability is more constrained than interspecific variability. We hypothesized that geographical variation is best explained by considering both local (habitat) and large-scale (climate) features.

First, populations with a restricted access to water should have lower TEWL rates and thermal preferences than those with permanent access after controlling for effects of the macrohabitat climate (Dupoué *et al.*, 2017b; Rozen-Rechels *et al.*, 2021). Second, populations from more arid environments should have lower TEWL rates, as seen in interspecific comparisons for reptiles (Cox & Cox, 2015; Garcia-Porta *et al.*, 2019), although it is unclear whether thermal preferences should increase or not with macrohabitat temperature and aridity. Metabolism at rest should be lower in warmer and more arid environments, as seen in recent broad-scale comparisons (Dupoué *et al.*, 2017a). Third, we expect inter-individual covariation between functionally related traits. For example, given that TEWL is the sum of respiratory (REWL) and cutaneous evaporative water losses (CEWL), we expect a positive correlation between TEWL and CEWL measured in similar conditions in the same animals, and also a correlation between TEWL and oxygen consumption (VO_2), because REWL scales linearly with VO_2 (Pirtle *et al.*, 2019).

MATERIAL AND METHODS

Study species

The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae) with a wide Eurosiberian distribution ranging from Southern France and Central Europe to Japan on Hokkaido Island (Surget-Groba *et al.*, 2006). It occupies cold and wet habitats including open heat lands, humid grasslands and peat bogs, in addition to clearances and clear-cuts surrounded by forests. Most European populations are viviparous, except for some oviparous lineages distributed at the south-western margin of the range in France and in Southern Central Europe (Surget-Groba *et al.*, 2001). We studied the oviparous south-west European common lizard, *Z. vivipara louislantzi*, which consists of four major subclades and inhabits a range of habitats from sea level to highlands (Milá *et al.*, 2013). We focused our field sampling on clade B2 in France to avoid interpopulation differences attributable to genetic differentiation between clades with potential introgression (Milá *et al.*, 2013; Dupoué *et al.*, 2021).

Sampling design and sampling site

In a recent study of the range distribution of this subspecies in south-western France, we found that temperature, rainfall and forest cover shape demographic and genetic variability from the rear to the leading edges (Dupoué *et al.*, 2021). We subsampled sites within this geographical range to select 15 populations along a sharp climate gradient for both temperature and rainfall, and we contrasted habitat features including differences in access to free-standing water and in forest cover. These variables adequately quantify aspects of the thermal and hydric gradients that should be relevant, considering previous findings showing interactive effects of hydric and thermal conditions on thermoregulatory behaviour (Rozen-Rechels *et al.*, 2021). In addition, geographical variation of some morphological and reproductive traits is correlated with habitat humidity and rainfall in this species (Lorenzon *et al.*, 2001; Marquis *et al.*, 2008; Dupoué *et al.*, 2017b).

To select our focal populations, we gathered elevation, habitat data and climate information for ~130 known occurrence sites in south-western France (Cistude Nature and Nature en Occitanie, unpublished data). For each site, we calculated the average temperature

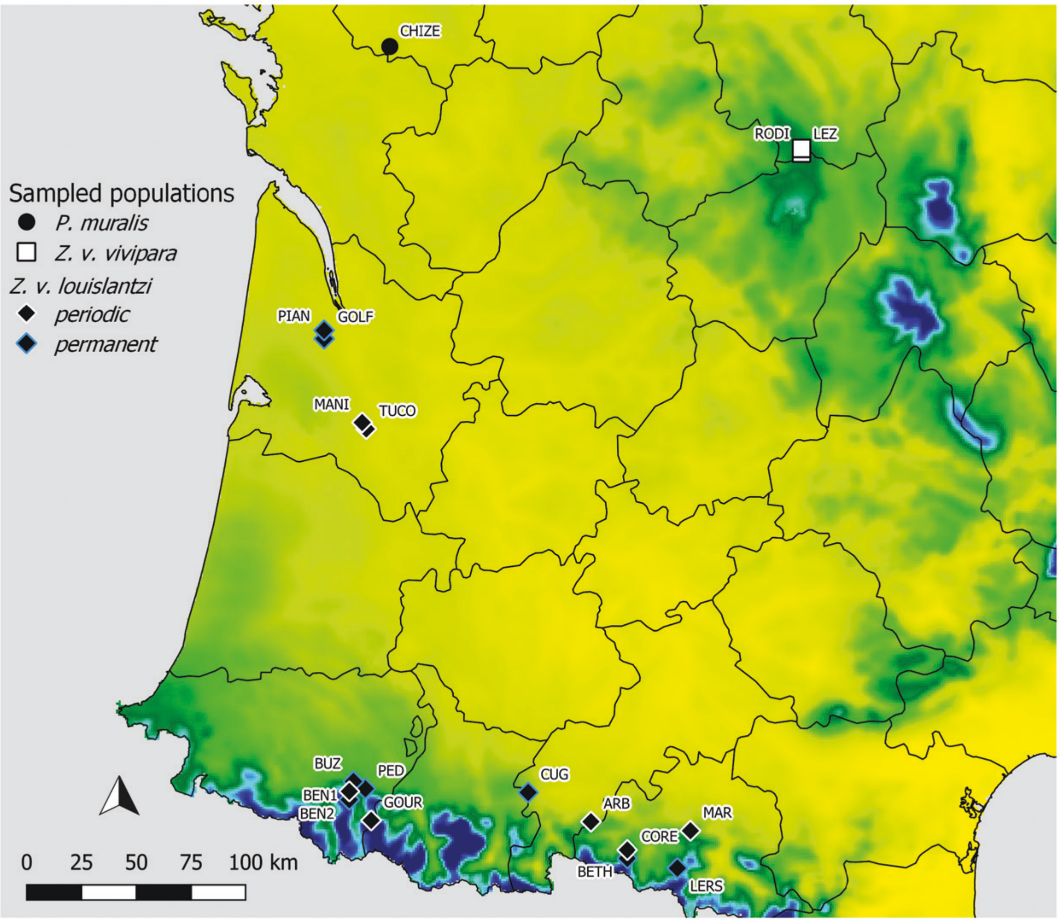

Figure 1. Map of populations sampled for *Zootoca vivipara louislantzi* (clade B2) in south-western France at different pluviometry quotient levels (background colours, yellow to blue gradient from low to high pluviometry) and with permanent (diamond with blue outline) or periodic (diamond with white outline) access to water. Two outgroup populations of *Zootoca vivipara vivipara* (LEZ and RODI) and one of *Podarcis muralis* (CHIZE) were included for comparative analyses.

and the cumulative precipitation during the activity season (from April to September) using 30 arcsec resolution climatic data derived from AURELHY climatic model outputs developed by Meteo-France (averages from the 1971–2000 period of weather survey; Canellas *et al.*, 2014). We also extracted the minimum temperature of the coldest month (T_{cold}) and the maximum of the hottest month (T_{hot}) and the annual precipitation P, to calculate the annual pluviometry quotient, $Q = (100 \times P)/(T_{hot}^2 - T_{cold}^2)$, also called the Emberger index (Emberger, 1955). This parameter allows discrimination of mesic (high Q index) from arid (low Q index) climates.

We then selected 15 representative populations of *Z. vivipara louislantzi* (clade B2) distributed along the elevational gradient with different temperatures (maximum temperatures during activity period ranging from 20 to 26 °C), rainfall levels (total precipitation during activity period ranging from 360 to 850 mm) and access to free-standing water at the same elevation (Fig. 1; Supporting Information, Table S1). Map was plot using QGIS software version 3.10.9-A Coruña with the perimeters of the French departments in the background. These populations were also associated with an elevational gradient (40–1750 m) and with a variety of habitats, including: (1) sites with permanent access to free-standing water (presence of water sources available to the lizards throughout the year, such as lake, streams and peat bogs) vs. temporary access to free-standing water (water body present only after rain or during the wet season; see Dupoué *et al.*, 2017b); and (2) sites with forest cover vs. open habitats, calculated with a forest cover index detailed by Rutschmann *et al.* (2016). We chose to sample populations with as much contrasted access to water and forest cover along the elevational and aridity gradients as possible, and then there was no significant correlation between mean temperature or mean rainfall and water access, but a small and significant correlation (Kruskal–Wallis test = 5, d.f. = 1, $P = 0.02$) between temperature or rainfall and forest cover.

For comparative purposes, we sampled two populations of the viviparous *Z. vivipara vivipara* clade E1 from the nearest locations in the Limousin area at ~800 m a.s.l. (Fig. 1). We also sampled wall lizards (*Podarcis muralis*) from a lowland population in the same area (Chizé population, 46°08′51.5″N, 0°25′36.4″W, ~64 m a.s.l.), which fell within the climatic range of the common lizard populations, in order to limit geographical effects. This widespread species is adapted to more arid environments and served us as an outgroup to compare with the common lizard, which is adapted to mesic and cold environments (Fig. 1). We attempted to capture about ten adult males per population (Supporting Information, Table S2).

Oviparous populations from south-western France are characterized by a strong seasonal activity pattern, sexual maturation at the age of 1–2 years old, facultative multiple clutches per year, and with an adult snout–vent length (SVL) ranging from 45 to 75 mm in males (Heulin, 1987; Heulin *et al.*, 1997). Sexually mature males were characterized by body coloration, the presence of femoral pores and the shape of the hemipenis. Given that sexually mature males are most likely to emerge earlier during the season in warmer environments, lowland populations were sampled first to reduce differences in reproductive phenology among populations (from 17 April 2019 in the lowland populations until 7 May 2019 in the highlands).

FIELD MEASUREMENTS AND HOUSING

We captured individuals by hand and brought them back to a laboratory (CEBC, CNRS, Villiers-en-bois, France) within 2–4 days after capture. The age class of sexually mature males was scored unambiguously using external morphology to separate young adults (aged < 1 calendar year and born in 2018) from older adults (aged ≥ 2 calendar years). One person (J.-F.L.G.) then measured SVL and total length to the nearest millimetre with a plastic ruler and body weight to the nearest milligram with an electronic balance.

The ventral and dorsal surfaces of all males were scanned at 600 dots per inch using a flatbed scanner (ScanJet 3670, Hewlett-Packard; see Brusch *et al.*, 2020) with animals evenly laid flat on the scanner on their belly and back using a foam cushion, to measure body surface and tail width, given that body area is a crucial determinant of total water loss. To do so, we processed the digital images with the software IMAGEJ (Schneider *et al.*, 2012) using the Analyze Particles function to calculate the body area. We first determined a threshold in terms of the colour of pixels to tell the lizard apart from the background, then the software automatically counted the number of pixels corresponding to the lizard body area. We repeated the analysis with ventral and dorsal scans of each lizard and summed the two areas. Tail width was measured at the seventh, tenth and 12th subcaudal scales and at maximum width (Brusch *et al.*, 2020). Scans were not always exploitable for this measurement, with 124 usable values out of 142 for the tenth and 12th subcaudal scales, 117 for the seventh subcaudal and 94 for the maximum width.

On the day of capture, we took a blood sample from the postorbital sinus using one or two micro-capillary tubes (~20–30 µL whole blood) and kept samples at ~4 °C in a cooler. We used the blood samples to assess how plasma osmolality (one of the best proxies for physiological dehydration in lizards; see Peterson, 1996) varied among populations and whether the lizards could maintain normosmolality in the face of strong variation in environmental conditions (Dupoué *et al.*, 2017b). In the laboratory, samples were centrifuged at 9500 g for 5 min to separate plasma from red blood cells. Plasma samples (~5–15 µL) were immediately frozen at −28 °C until the osmolality assays. Later, plasma osmolality was determined using a vapour pressure osmometer (model Vapro 5600, ELITechGroup) with the protocol described by Wright *et al.* (2013) and adjusted to small plasma volumes (Dupoué *et al.*, 2017b). Before analyses, plasma was diluted (1:3) in standard saline solution (osmolarity = 280 mOsm L^{-1}) to obtain two duplicates per sample. Thus, we were able to estimate an intra-assay coefficient of variation (CV < 3%).

Animals were kept separated in individual terraria (35 cm × 25 cm × 12.5 cm) with peat soil, a PVC tube for shelter and a water cup for permanent access to free-standing water. During captivity, we provided all lizards with the same thermal gradient from 20 to 34 °C for 8 h per day (09:00–17:00 h) using heating wires placed under one end of each terrarium. The lizards were all fed with live crickets (*Acheta domesticus*) every 2 days and had water sprayed three times a day in addition to the water available *ad libitum*. Physiological assays and behavioural trials started after a 1-week acclimation period (see below). Once all measurements were completed, all males were released at the capture location.

ECOPHYSIOLOGICAL MEASUREMENTS

We separated animals from different populations randomly into 12 lots of 15 lizards. Then, each lot went through the same succession of laboratory measurements as summarized in the Supporting Information (Fig. S1). Individuals were maintained without food for 4 days and weighed with a high-precision balance (body mass ±0.01 mg) at 20:00 h at the end of day 3. They were then placed overnight in an open-topped plastic box with no water or food, inside a climatic chamber set at 20 °C and 60% relative humidity. All lizards were reweighed at 08:00 h the following day (day 4) and moved back to their individual terrarium. We assessed total evaporative water loss (TEWL) from the loss of mass (in milligrams per hour) after discounting individuals that had defecated during the night ($N = 27$). In squamate reptiles, body mass loss reflects TEWL (i.e. the sum of ventilatory and cutaneous evaporative water losses) in resting animals, because variation in body mass is mostly attributable to water loss (Moen *et al.*, 2005; Dupoué *et al.*, 2015). We also measured body mass loss at rest during metabolic rate assays on day 6 (Supporting Information, Fig. S1) and found a strong correlation between these two measurements of TEWL, showing that this protocol was highly repeatable (repeatability estimation using the linear mixed-effects models method including animals that did not defecate during the tests, $N = 59$, $r = 0.64 \pm 0.08$, $P < 0.0001$).

The next day (day 5), we measured the T_{pref} of fasted lizards in a photothermal gradient inside a neutral arena where water was available *ad libitum* and the lizards were safe from predators and competitors (Artacho *et al.*, 2013). Around noon, lizards were placed individually in a 60-cm-long plastic box with a 40 W light bulb at one end and a water cup (Trochet *et al.*, 2018). By maintaining the air in the room at 18 °C, we could simulate a thermal gradient ranging from 18 to 43 °C below the light bulb. Experiments started ~13:00 h, and we collected data after an acclimation period of ≥ 30 min. Every 20 min during 3 h 30 min, we measured the surface temperature of the lizard on the back with a high-precision infrared thermometer (Raynger MX2, Raytek). We collected 11 repeated measurements for each individual to assess T_{pref} values. Differences in thermal preferences between populations were then analysed using all observations except those of animals buried in the soil, which we considered irrelevant because lizards were 'inactive'. At the end of the day, we also measured the core body temperature of 120 individuals (randomly chosen among types and populations) with the tip of a K-type temperature probe (Hanna HI 935002, Hanna Instruments) inserted in the cloaca. This allowed us to calculate a calibration curve between surface temperature and core body temperature. We found a highly significant, close to 1:1 relationship between core body temperature and surface infrared measurements for the three taxonomic groups of lizards (major axis regression forced to zero, slope = 0.96 ± 0.01, $P < 0.0001$, $r^2 > 0.99$). We used this regression to convert all surface temperatures to core body temperatures for further analyses.

On day 6, we used the indirect calorimetric method with a closed respirometer system described by Foucart *et al.* (2014) to measure resting metabolic rate from the CO_2 and O_2 gas exchange between animals and the atmosphere. Lizards fasted for 6 days were weighed with the high-precision scale, then placed in opaque test chambers (1 L) covered with air-tight glass lids. Chambers were installed in a temperature-controlled environment (set at 20 °C, a temperature that does not elicit significant locomotor activity) 2 h before beginning the trial. Outside air was drawn into each test chamber using a Bioblock Scientific 55 L air pump for 5 min ensuring ≥ 99% air turnover. Baseline samples of outside air were collected at the onset of each trial. Trials were performed during the night (12 h), meaning that the lizards were inactive most of the time. At the end of each trial, an air sample was collected from inside the chambers using two 150 mL syringes, and we noted whether the lizards had defecated during the night ($N = 41$).

The baseline and final CO_2 and O_2 concentrations were determined using high-precision gas analysers (CA10 and FC10A, Sable Systems, Las Vegas, NV, USA). Air was pushed from the syringes using an infusion pump (KDS210, KD Scientific, Holliston, MA, USA), passed through Drierite to remove water, then sent at a controlled rate to the analyser, which was calibrated before each trial. With baseline and final concentrations, we calculated the VO_2 (volume of O_2 consumed, in millilitres per hour) and the respiratory quotient as the ratio of CO_2 produced to O_2 consumed. The respiratory quotient varied around 0.75 (mean = 0.77 ± 0.032, range = 0.695–0.911), which corresponds to the situation of a fasting animal catabolizing lipids (Schmidt-Nielsen, 1997). Subsequently, we analysed resting metabolic rate using VO_2 values.

On day 7 the fast was ended, and on day 8 we used an evaporimeter (Aquaflux BIOX AF200, Biox Systems, UK) to quantify the transepidermal water vapour flux from measurements of a humidity gradient within a closed chamber (Imhof *et al.*, 2009; Guillon *et al.*, 2013). We made independent measurements of the cutaneous evaporative water loss (CEWL rate, in grams of water per square metre per hour) from four locations on the back of each lizard in a laboratory room maintained at 23 °C. We used an *in vivo* nail cap with a rubber O-ring (diameter 2.6 mm) to ensure a complete seal between the measurement chamber and the skin of the lizard. The evaporimeter was calibrated at the beginning of each trial according to the manufacturer's procedures. We then gently pressed the probe against the skin of the lizard for a few minutes to perform measurements.

Each measurement trial ended once the real-time CEWL reading, monitored on a graphical interface, was stable for 10 s (±0.01 g m^{-2} h^{-1}). If any movement caused a leak in the seal between the measurement chamber and the skin of the animal, we repeated the trial. In total, we performed four measurements for each animal and evaluated their quality a posteriori according to the shape of the curve, as recommended by the manufacturer. We retained only good-quality measurements for subsequent analyses (range = 2–4 per lizard, mean = 3.66). The repeatability for measurements on the same individual was estimated using the linear mixed-effects models method and was significant ($r = 0.56 \pm 0.04$, $P < 0.0001$).

STATISTICAL ANALYSES

All statistical analyses were performed in R software v.3.5.3 (2019-03-11) 'Great Truth' (R Core Team, 2019). In our results, we provide the standard deviation of the mean as the dispersion measure (mean ± SD). In a first set of analyses, we compared measurements obtained in the two clades of common lizards and the wall lizards (*Z. vivipara louislantzi*, *Z. vivipara vivipara* and *P. muralis*) with an ANOVA. For each measurement, we performed an ANCOVA test, controlling for inter-individual differences in body mass. In a second set of analyses, we studied geographical variation among oviparous populations of *Z. vivipara louislantzi*. To test for potential correlations between morphological, behavioural and physiological traits on the one hand and the population characteristics on the other, we used a two-step model selection procedure. We analysed independently the variation of body size (SVL, in millimetres), body mass (W, in grams), body surface area (in square millimetres), thermal preferences (T_{pref}, in degrees Celsius), total water loss (TEWL, in milligrams per hour), cutaneous water loss (CEWL, in grams of water per square metre per hour) and resting metabolic rate (VO_2, in millilitres per hour) using linear mixed models with the function lme from the package nlme (Pinheiro *et al.*, 2006). For each variable, we followed the same model selection procedure (as shown in Supporting Information, Tables S3–S11).

Given that our variables of interest were influenced by both individual-level and population-level factors, we proceeded in two steps. First, we fitted a full model, including all individual and experimental covariates as fixed effects and selected a first minimum adequate model using a backward model selection based on the Akaike information criterion (AIC). We also assessed homoscedasticity and normality of residuals using the full model. Experimental covariates included measurement conditions (time of the day, temperature and humidity in the room when the experiment was not performed in climatic chambers), and the presence of faeces for TEWL and VO_2. Individual covariates included age class, SVL, body surface area and/or body mass, depending on traits (see details below and in the Supporting Information, Tables S3-S11). Full models included additive linear effects of all covariates. For thermal preference data, we analysed all body temperatures recorded for each individual rather than a composite variable (e.g. mean or variance). Population identity was always included as a random effect to control for non-independence among individuals from the same population. Individual identity nested in the population identity was also included as a random effect to account for repeated measurements on the same individual for T_{pref} and CEWL data.

In a second step, we tested whether traits differed significantly among populations owing to differences in climate or habitat. We used a model-averaging procedure using the AIC comparison procedure (Burnham & Anderson, 2004) to compare the relative importance [sum of AIC corrected for small sample size (AICc) over all models in which the variable appears] of environmental variables related to elevation, climatic conditions (mean temperature and rainfall during activity season, pluviometry quotient) and habitat features (water presence and forest cover). The model-averaging procedure was performed with maximum likelihood estimates of model parameters using the MuMIn package (Burnham & Anderson, 2002; Barton & Barton, 2015). Models were constructed from the best model selected during the first step described above, in which we added the environmental variables alone as a fixed effect or the additive effects of one variable describing climatic conditions and one variable describing habitat features. Correlations between the environmental variables and the habitat variables were low, because we chose to sample populations with as much contrasted access to water and forest cover along the elevation and aridity gradients as possible. For the purpose of model-averaging calculations, all continuous covariates were centred and scaled, and categorical covariates (age, water presence and forest cover) had their contrasts summed to zero. We selected the main effects with significant contributions to the variability of the traits based on the AICc difference between models, the relative weight of the models and the importance, in addition to conditional averages from the model-averaging procedure (Burnham & Anderson, 2004). If the minimal model was included in the set of best models, this implied uncertainty in the importance of environmental variables. If numerous concurrent best models were observed, this implied uncertainty about which environmental variable explained the most geographical variation in the trait. All model-averaging tables and conditional averages of the important environmental variables are reported in the Supporting Information (Tables S3–S11).

To complement these independent analyses of each trait, we also performed a path analysis with piecewise structural equation modelling using the R package PIECEWISESEM (Lefcheck, 2016). This procedure allowed us to test causal paths identified in our model-selection approach in a single model accounting for the multivariate relationships between our interrelated traits (for more details, see Supporting Information, Appendix S1).

RESULTS

DIFFERENCES BETWEEN TAXA

We found significant differences for all thermo-hydroregulation traits between the wall lizards and the two groups of common lizards, but not between oviparous and viviparous populations of the common lizard, even after controlling for the allometric relationship (Table 1; for details, see Supporting Information, Table S2). The TEWL was 40% lower in *P. muralis* than in the two clades of *Z. vivipara* after controlling for body mass (*P. muralis*, TEWL = 0.853 mg g^{-1} h^{-1}; *Z. vivipara louislantzi*, TEWL = 1.387 mg g^{-1} h^{-1}; *Z. vivipara vivipara*, TEWL = 1.489 mg g^{-1} h^{-1}; ANCOVA: taxa, $F_{2,140} = 10.9$, $P < 0.0001$; body mass, $F_{1,140} = 77.2$, $P < 0.0001$). Likewise, CEWL was 40% lower in *P. muralis* than in the two clades of *Z. vivipara* (ANOVA: taxa, $F_{2,652} = 66.3$, $P < 0.0001$). The VO_2 rates, corrected for body mass, were 26% lower in *P. muralis* than in *Z. vivipara* (ANCOVA: body mass, $F_{1,160} = 336.6$, $P < 0.0001$; taxa, $F_{2,160} = 9.63$, $P = 0.0001$). The values of T_{pref} were significantly lower in *P. muralis* compared with the *Z. vivipara* subspecies (ANOVA: $F_{2,1640} = 47.19$, $P < 0.001$). None of the post hoc tests revealed any difference between *Z. vivipara louislantzi* and *Z. vivipara vivipara* (Table 1).

MORPHOLOGICAL VARIATION AMONG OVIPAROUS POPULATIONS OF *Z. VIVIPARA*

The SVL and body condition (body mass corrected for SVL with a linear regression) varied among populations (ANOVA: population effect, $F_{14,127} = 4.7$, $P < 0.0001$ and $R_{interpop} = 0.38$ for SVL; and ANOVA: population effect, $F_{14,126} = 7.0$, $P < 0.0001$ and $R_{interpop} = 0.42$ for body mass), and these differences were explained, in part, by climatic conditions. The most important environmental and individual variables explaining the variation of SVL included the age class (contrast between males aged 1 year and older males = −3.2 ± 0.25 mm, $P < 0.0001$), mean temperature during the activity period (slope = −0.5 ± 0.16 mm °C^{-1}, $P = 0.01$) and the pluviometry quotient (slope = 0.01 ± 0.005 mm per Q unit, $P = 0.04$; see Supporting Information, Table S3). Thus, lizards had a larger average body size in colder climates (Fig. 2A). The most important environmental variables explaining geographical variation of body condition were the pluviometry quotient (slope = 0.002 ± 0.001 g per Q unit, $P = 0.03$), the elevation (estimate = 0.0003 ± 0.0001 g m^{-1}, $P = 0.03$) and the mean temperature during the activity period (estimate = -0.07 ± 0.03 g °C^{-1}, $P = 0.04$). According to the best-supported model, lizards had a higher body condition in highland populations with a high pluviometry quotient and in open habitats rather than under dense forest cover (see variations of body mass in Fig. 2B; Supporting Information, Table S4). Body surface area was, as expected, strongly correlated with body mass ($r = 0.9$, $P < 0.0001$) and followed a similar pattern of geographical variation to body condition (see Supporting Information, Table S5).

Tail width at the three recorded positions was correlated with SVL and body mass, and the different measurements of tail width were strongly correlated with each other (all $r > 0.75$, $P < 0.0001$). We present only the analyses on maximal tail width and tail width at the tenth subcaudal scale. For both of these

Table 1. Mean body mass, water loss rates, oxygen consumption rates and thermal preferences in the two subspecies of *Zootoca vivipara* and in *Podarcis muralis*

Parameter	***Zootoca vivipara louislantzi***	***Zootoca vivipara vivipara***	***Podarcis muralis***
Body mass (g)	2.90 ± 0.66	3.15 ± 0.75	5.26 ± 1.60
TEWL (mg h^{-1})	3.63 ± 0.78	3.97 ± 0.68	4.08 ± 1.70
TEWL per mass (mg h^{-1} g^{-1})	1.42 ± 0.29	1.54 ± 0.27	0.87 ± 0.24
CEWL (g m^{-2} h^{-1})	16.92 ± 4.35	17.86 ± 3.11	10.59 ± 2.23
VO_2 (ml h^{-1})	0.186 ± 0.03	0.203 ± 0.03	0.254 ± 0.06
VO_2 per mass (ml h^{-1} g^{-1})	0.075 ± 0.013	0.073 ± 0.012	0.058 ± 0.008
T_{pref} (°C)	34.94 ± 2.44	34.67 ± 2.75	32.08 ± 3.51

Values are the means (±SD) for 142 *Z. vivipara louislantzi*, 21 *Z. vivipara vivipara* and 17 *P. muralis* adult males.
Abbreviations: CEWL, cutaneous evaporative water loss; TEWL, total evaporative water loss; T_{pref}, preferred body temperature; VO_2, oxygen consumption.

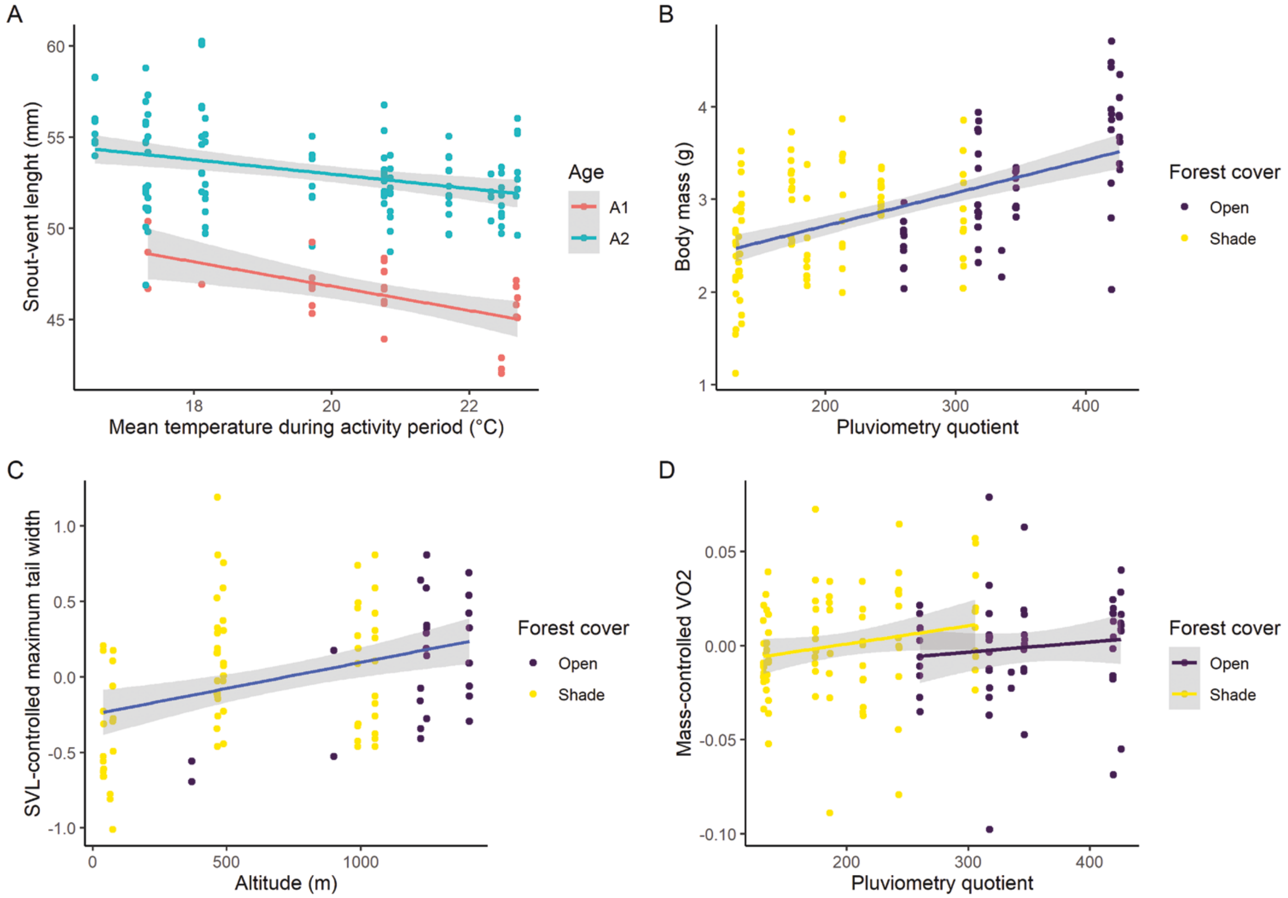

Figure 2. Spatial variation of morphological and physiological traits along an environmental gradient. A, snout–vent length (SVL) variation along a gradient of mean environmental temperature during the activity period; colour code represents the age class of the lizards (A1, 1 year old; A2, > 1 year old). B, body mass variation along a gradient of aridity of the environment (low values indicate less mesic environments); colour separates forest-covered habitats (yellow) from open habitats (purple). C, maximal tail width (in millimetres) relative to SVL (residuals of a linear regression) along a gradient of elevation; colour separates forest-covered habitats (yellow) from open habitats (purple). $N = 94$ owing to missing values. D, volume of oxygen consumed (VO_2, in millilitres per hour) relative to the body mass (residuals of a linear regression) according to the aridity of the environment; colour separates forest-covered environments (yellow) from open environments (purple).

measurements, the most important environmental and individual variables included the SVL (for maximal width, slope = 0.046 ± 0.021 mm, $P = 0.03$; for width at tenth scale, slope = 0.074 ± 0.015 mm mm^{-1}, $P < 0.0001$), the elevation (for maximal width, slope = 0.0006 ± 0.0002 mm m^{-1}, $P = 0.0009$; for width at tenth scale: slope = 0.0005 ± 0.0001 mm m^{-1}, $P = 0.0003$; see Fig. 2C; Supporting Information, Table S6), the mean temperature during the activity period (for maximal width, slope = –0.11 ± 0.04 mm °C^{-1}, $P = 0.018$; for width at tenth scale, slope = –0.09 ± 0.03 mm °C^{-1}, $P = 0.006$) and the mean precipitation during the activity period (for maximal width, slope = 0.001 ± 0.0007 mm mm^{-1}, $P = 0.046$; for width at tenth scale, slope = 0.001 ± 0.0005 mm mm^{-1}, $P = 0.03$). The best-supported models retained the effect of age class and SVL as individual covariates and of elevation and forest cover, indicating a joint effect of climate conditions and local habitat (Fig. 2C; Supporting Information, Table S6).

PHYSIOLOGICAL VARIATION AMONG OVIPAROUS POPULATIONS OF *Z. VIVIPARA*

The TEWL at rest increased with body mass (estimate = 0.0008 ± 0.0001 mg h^{-1} g^{-1}, $P < 0.0001$). There was some degree of variation in TEWL among populations (ANOVA: population effect, $F_{14,94} = 3.5$, $P = 0.0001$ and $R_{\text{interpop}} = 0.37$), and our model comparison procedure uncovered that the permanent access to free water in the habitat was a significant variable explaining this geographical variation

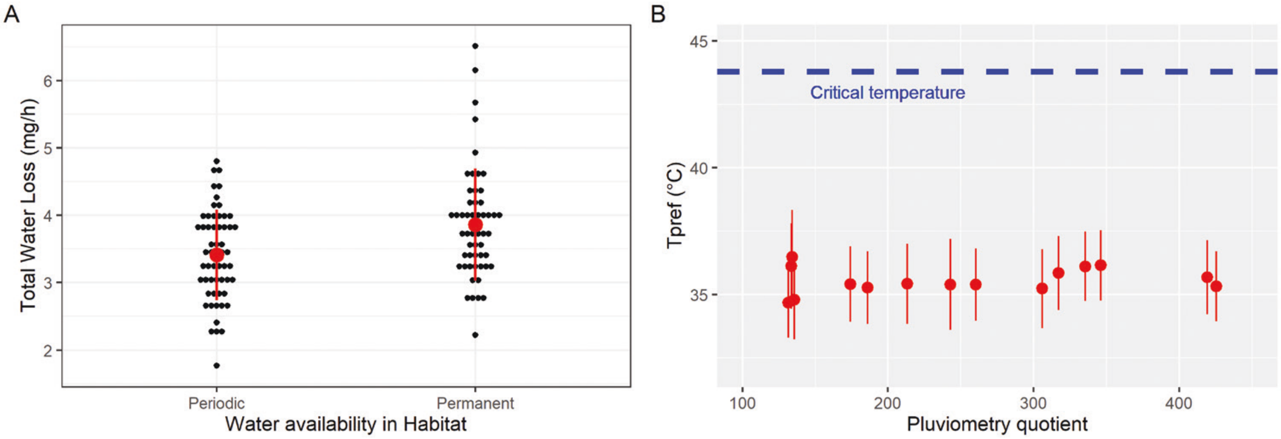

Figure 3. Variation in oviparous populations of *Zootoca vivipara*. A, rates of total evaporative water loss according to the availability of water in the habitat; red dot is the mean value and red lines are standard deviations. B, mean and standard deviation of preferred body temperature (T_{pref}) across populations along a gradient of aridity of the environment (low values indicate less mesic environments). The blue dashed line is the critical thermal maximum of *Z. vivipara* (Gvoždík & Castilla, 2001).

(Fig. 3A; Supporting Information, Table S7). Lizards living in habitats with no permanent access to water had lower TEWL (contrast = −0.26 ± 0.07, $P = 0.001$). The effects of other variables were more uncertain, with models suggesting that TEWL decreased weakly with elevation and in open habitats compared with forest habitats (Supporting Information, Table S7). The CEWL values showed a much greater variability among individuals within a population than among populations ($R_{interpop} = 0.22$; $R_{interind} = 0.47$). The model that best explained CEWL variation included only effects of experimental conditions, with a positive correlation for the absolute humidity of the room at the time of measurement (estimator = 0.70 ± 0.04, $P = 0.0001$; Supporting Information, Table S8).

Apart from the significant positive effect of body mass on VO_2 at rest (estimate = $6.14 \times 10^{-5} \pm 5 \times 10^{-6}$ ml h^{-1} mg^{-1}, $P < 0.0001$), there was some uncertainty about the effects of environmental variables on VO_2, despite variation among populations (ANOVA: population effect, $F_{17,144} = 1.91$, $P = 0.02$ and $R_{interpop} = 0.25$). According to the best-supported model, lizards tended to have higher VO_2 in populations with a high pluviometry quotient and, in addition, in forest habitats compared with open areas (Fig. 2D; Supporting Information, Table S9).

Mean plasma osmolality was 306 ± 18 mOsm kg^{-1} and was different among populations (ANOVA: population effect, $F_{14,119} = 3.8$, $P < 0.0001$ and $R_{interpop} = 0.35$), but we found no significant effect of environmental variables on plasma osmolality (Supporting Information, Table S10).

VARIATION OF THERMAL PREFERENCES IN OVIPAROUS POPULATIONS OF *Z. VIVIPARA*

Thermal body preferences were relatively high in our sample (mean = 34.94 °C, range = 19.80–39.40 °C; Fig. 3B) in comparison to previous studies with the same species (e.g. $T_{pref} = 31.5$ °C; Gvoždík & Castilla, 2001; Trochet *et al.*, 2018). The T_{pref} showed a great intrapopulation variability but varied little across populations (ANOVA: population effect, $F_{14,1420} = 5.11$, $P < 0.001$ and $R_{interpop} = 0.18$; Fig. 3B), and this geographical variation was not significantly explained by environmental variables. Our model comparison procedure only revealed the effect of experimental covariates and suggested some potential but weak effects of forest cover (not significant in conditional averages; Supporting Information, Table S11). Lizards living in open habitats tended to have higher T_{pref} than lizards from forest habitats.

COVARIATION BETWEEN THERMO-HYDROREGULATION TRAITS

We found no significant correlation between TEWL, CEWL, VO_2, the body surface area of the animal and the T_{pref} (Fig. 4). First, TEWL, T_{pref} and VO_2, which provide three potentially related types of information about the thermo-hydroregulation strategy of lizards, were not strongly correlated (Pearson product–moment correlation, all $r < 0.3$, all $P < 0.05$). Second, we explored the relationship between TEWL and the potential subcomponents of evaporative water loss, such as CEWL, body surface area and ventilation rate (which is linearly related to VO_2), but we found no correlation between them (all $r < 0.2$, all $P > 0.05$). In

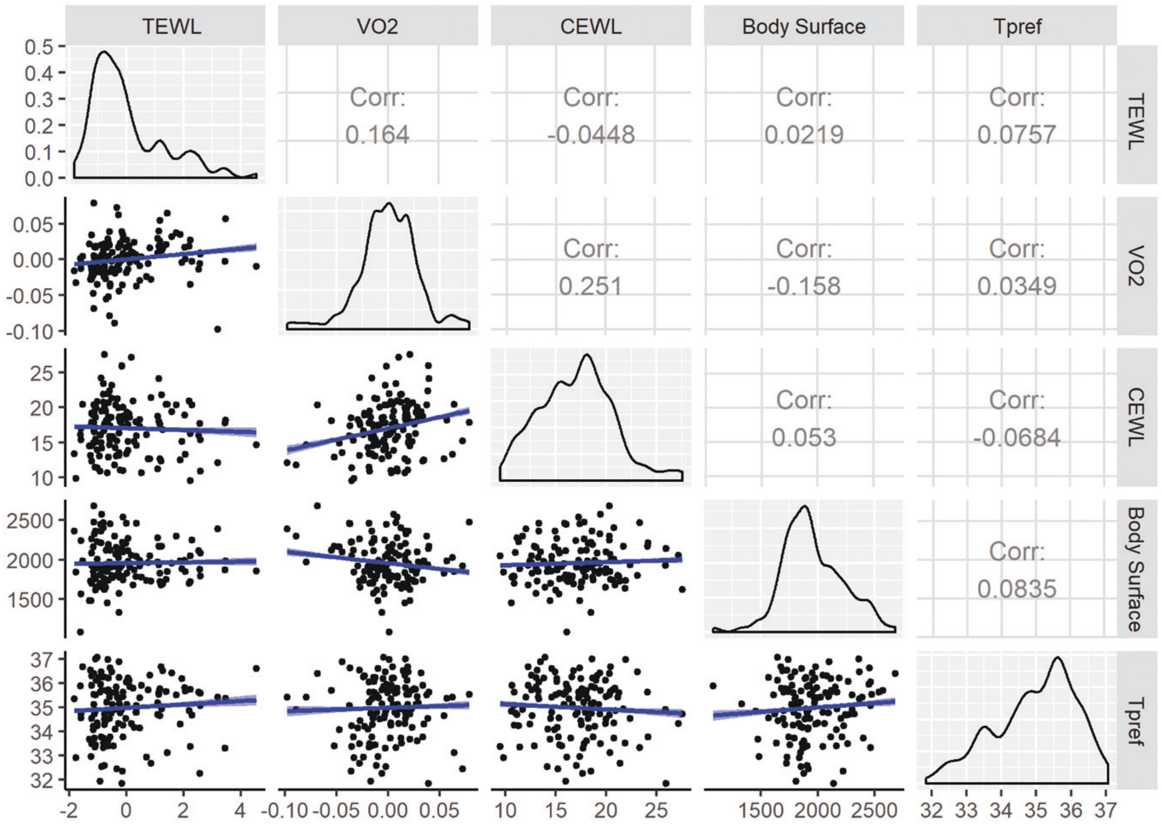

Figure 4. Scatterplot matrix of total and cutaneous water losses [TEWL (in milligrams per gram per hour) and CEWL (in grams per square metre per hour)], metabolic rate [VO_2 (in millilitres per hour)], total body surface area (in square millimetres) and preferred body temperature [T_{pref} (in degrees Celsius)]. The diagonal represents the distribution of each variable, the upper triangle provides the Pearson correlation coefficients between pairs of variables, and the lower triangle provides scatter plots of each pair of variables with the best linear regression line.

addition, in the structural equation modelling result, CEWL and VO_2 exhibited a weakly positive correlation ($r = 0.26$, $P = 0.018$; see Supporting Information, Appendix S1). Otherwise, model comparison with AIC gives similar results to those of the piecewiseSEM procedure (see Supporting Information, Appendix S1).

DISCUSSION

Studies of intraspecific variation in functional traits across environmental gradients have tended to focus on the thermal biology of ectotherms instead of the joint variation and integration of their thermo-hydroregulation strategies (Artacho *et al.*, 2017; Domínguez-Guerrero *et al.*, 2021). Here, we compared ecophysiological traits linked to both water balance and body temperature regulation in populations of *Z. vivipara louislantzi* ranging from the rear edge near sea level to the leading edge of their distribution in highland habitats. In addition, we compared these populations with two outgroups, a different species and another subspecies, from which we expected substantial ecophysiological differences.

Strong ecophysiological variation was observed between the wall lizards (*P. muralis*) and common lizards (*Z. vivipara*), as expected given the broad ecological and habitat differences between these two species. The wall lizard is found, on average, in warmer and drier areas than the common lizard, and this species also exhibits distinct preferences for dry saxicolous rather vegetated habitats where common lizards thrive, although these two species can occur sympatrically (Mole, 2010). On the one hand, *P. muralis* had lower water loss rates (both TEWL and CEWL) and basal metabolic rates (VO_2) than *Z. vivipara*. Comparative studies of squamate reptiles and other vertebrates have uncovered similar findings, with higher resistance to evaporative water loss and lower basal energy expenditure in species from hot and dry environments (Cox & Cox, 2015; Dupoué *et al.*, 2017a; Le Galliard *et al.*, 2021a). On the other hand, *P. muralis* displayed lower preferred temperatures (T_{pref}) than our populations of *Z. vivipara* instead of the predicted higher T_{pref} expected for organisms from warmer habitats (Garcia-Porta *et al.*, 2019). Previous studies of *P. muralis* and *Z. vivipara* have found that the T_{pref} of adult males can vary seasonally, with high T_{pref} during the early mating season, lower T_{pref} during the spring season and higher T_{pref} during the summer season (Osojnik *et al.*, 2013). Thus, one possibility is that wall lizards, which tend to have an earlier phenology than common lizards, had lower T_{pref} because they were sampled slightly later during their mating season than common lizards. Another possibility is that the relatively lower T_{pref} of wall lizards reflects adjustments to water limitation and habitat aridity in their native population, as shown in laboratory experiments (Sannolo & Carretero, 2019; Le Galliard *et al.*, 2021b).

We found no significant ecophysiological variation between the two clades of *Z. vivipara* despite their ancient evolutionary divergence and a major difference in reproduction mode between the two clades. Phylogenetic studies revealed six major genetic clades for *Z. vivipara* in Eurasia, with four widespread viviparous clades present in most of the distribution range and two oviparous clades restricted to the southern margin of the range distribution in Western or Eastern Europe (Surget-Groba *et al.*, 2006). The most parsimonious evolutionary scenario proposes that viviparity evolved only once but that a reversal to oviparity occurred in the western populations from our study area (Horreo *et al.*, 2018; Recknagel *et al.*, 2018). This transition back to oviparity was associated with climate warming, because oviparous forms are generally favoured over viviparous forms in warmer habitats (Shine, 1985). The two reproductive modes are probably separated, given the limited gene flow and reproductive isolation between them (e.g. Cornetti *et al.*, 2015). Viviparity is considered as a key evolutionary transition to life in cold climatic conditions (Horreo *et al.*, 2021), with oviparous lizards being adapted to a warmer and more variable environment during the reproductive season (as suggested by Horreo *et al.*, 2021). This scenario led us to expect major differences in thermo-hydroregulation strategies between clades B (oviparous) and E (viviparous). However, we found no physiological or behavioural adaptation, because the two clades were very similar in terms of TEWL, T_{pref} and VO_2. In our experiment, interindividual and interpopulation differences in these traits were stronger than the divergence attributable to clades. However, we encourage further studies of the ecophysiological strategies of adult females, especially during gestation, when phenotypic differences between the two reproductive modes are likely to be stronger (Recknagel & Elmer, 2019).

Morphological differences among oviparous populations were explained by climatic conditions, including temperature and the pluviometry quotient, *Q*. First, the body condition of males was explained by both environmental temperature and humidity: sexually mature males were, on average, heavier in the more mesic habitats (*sensu Q* index), whereas relative tail width increased with elevation (and cold temperatures) and with rainfall. In this species, caudal width represents a good proxy of tail reserves, which are an important body component for fat and protein storage, whose variation can reflect changes in food availability, energy intake and also water availability (Bateman & Fleming, 2009; Brusch *et al.*, 2020). Body condition might increase with humidity because of positive effects of rainfall and water availability on habitat quality, including food availability, but this should be accompanied by an increase of body size with humidity. Alternatively, low environmental temperatures and high water availability might reduce energy expenditure and the reliance of lizards on fat or protein catabolism to restore energy and water balance (e.g. Brusch *et al.*, 2020). Hence, we can hypothesize that males from rear edge, drier and hotter populations were more prompt to catabolize tail reserves, probably to fuel some higher metabolic demand (in warmer climates) and to support water demands (in drier climates). Further examinations of physiological traits in standard conditions tend to confirm the latter hypothesis.

Second, size was influenced mostly by environmental temperature: sexually mature males were, on average, longer in the colder habitats. This geographical variability for body size is globally consistent with previous data collected across the elevational range of other ectothermic vertebrates (Peterman *et al.*, 2016; Trochet *et al.*, 2019). These two aspects suggest a positive effect of cold temperature and humidity on body size and body condition. Although the effect on size has been documented previously (Lu *et al.*, 2018; Roitberg *et al.*, 2020), the effect of aridity is rarely tested. A recent thorough analysis of the body size variation across the range distribution of *Z. vivipara* suggested that body size varies non-monotonously with the duration of the activity season in this species because of the underlying thermal plasticity of growth and sexual maturation (Roitberg *et al.*, 2020). This scenario of thermal plasticity predicts a shift towards a larger mean body size of adult individuals in colder climates, because age at first reproduction is delayed by a year or more in a seasonal environment (Adolph & Porter, 1996). Thus, adult males are bigger in colder climates because they mature at an older age and therefore at a larger body size on average than adults growing at higher temperatures and maturing earlier in life (Atkinson, 1994; Angilletta, 2009). However, under this scenario, we would not expect the size of young males of the year (age class A1) to be smaller in warmer habitats if those yearlings are born earlier and grow faster than those in colder habitats (Sorci *et al.*, 1996; Roitberg *et al.*, 2020), which is what we found here. Our sample of 1-year-old individuals was, however, likely to be biased, because we only captured sexually mature young males, whereas most young are not mature at this age, especially in cold climates. We therefore performed a complementary analysis of a larger dataset including many more *Z. vivipara* populations and both sexually mature and immature young males of the year. In this supplementary database, we had a much stronger variability in the body size of young males of the year. This updated analysis confirmed a positive thermal cline for the body size of 1-year-old males, with SVL increasing by 1.03 ± 0.29 mm $°C^{-1}$ mean activity temperature ($P = 0.0006$; see Supporting Information, Table S12). This reverse thermal cline for body size in young males of the year and older males conforms exactly with the 'saw-tooth' relationship between body size and seasonality proposed by Adolph & Porter (1996) and seen across the broad geographical distribution of *Z. vivipara* across Europe (Roitberg *et al.*, 2020). Altogether, these results indicate that broad-scale climate gradients instead of local habitat features were the main determinants of geographical variation in morphology.

Lizards living in habitats with temporary access to water had lower TEWL rates than those from habitats with permanent access, and the plasma osmolality was remarkably consistent across habitat types and climate conditions, similar to a previous finding in adult males and females of the viviparous clade E in Massif Central, France (Dupoué *et al.*, 2017b). This confirms that common lizards can adjust or adapt their total water loss to cope with seasonal habitat dryness and that water availability in the environment is more consistently related to this functional trait of the water budget than rainfall or thermal conditions. We also examined traits involved in TEWL variation, including body surface area (which relates to the total exchange area for cutaneous evaporative water loss), standard cutaneous water loss rate (which relates to skin permeability to water loss) and VO_2 at rest (which relates to basal energy expenditure and is also correlated with respiratory water loss). Surprisingly, we found no effect of water availability in the habitat on these three traits and little inter-individual correlation between these traits and TEWL, which does not allow us to determine a specific route by which lizards downregulated standard TEWL rates in habitats with temporary access to water. One possibility is that some behavioural mechanisms were driving the observed pattern. For example, ocular water loss can represent a significant avenue for TEWL in small lizard species (Pirtle *et al.*, 2019), and the time spent with eyes open could be variable during inactivity periods.

Contrary to our expectations, the VO_2 of adult males was little influenced by climate conditions, especially environmental temperatures, except for a trend towards higher VO_2 in more mesic conditions that would require further testing with a larger sample size. Note that this weak pattern of variation of VO_2 with climate aridity is the same as the one seen in some desert species of birds and rodents (McNab & Morrison, 1963; Tieleman *et al.*, 2003). We also found a small positive effect of forest cover on VO_2, but this effect remains difficult to interpret given that closed habitats tend to be more frequent at a lower elevation. Given the strong and consistent differences in thermal and rainfall conditions between the relict lowland populations and those in highland mountains, we expected that the VO_2 of adult males would be significantly lower in low-elevation populations (i.e. metabolic cold adaptation or Krogh's rule, described by Krogh & Lindhard, 1914), as has been shown in other species of lizards (e.g. Plasman *et al.*, 2020). However, a recent review by Pettersen (2020) on reptiles demonstrated little support for counter-gradient variation in metabolic rate (which would reflect an adaptive response to geographical gradient), whereas it did reveal such an adaptive response in development time. Here, we did not find a difference in VO_2 at the adult stage across a 2000 m elevation gradient but cannot exclude the possibility that geographical variation might be more substantial at earlier stages of life or in females, but also in a different season, such as at the end of the summer, after seasonal acclimatization of resting metabolic rate.

Regarding thermoregulatory behaviour, we studied thermal preferences to quantify thermoregulation strategies in controlled laboratory conditions. Variation in T_{pref} was often higher among individuals within a population than among populations and fell 6–7 °C below the critical thermal maximum (Gvoždík & Castilla, 2001; Trochet *et al.*, 2018). Individual differences in T_{pref} were independent of variation in water loss rates or basal metabolism. The T_{pref} also remained similar, on average, among populations with contrasted climatic and habitat conditions, confirming the results of several previous comparative studies of thermal preferences in this species in other geographical areas (Van Damme *et al.*, 1990; Gvoždík & Castilla, 2001; Carretero *et al.*, 2005; Rozen-Rechels *et al.*, 2021). Note that we cannot exclude the possibility of larger geographical differences in T_{pref} later in the season owing to different acclimatization conditions across our geographical gradients, because a strong seasonality effect was found in a meta-analysis on thermoregulation efficiency of lacertid lizards (Ortega & Martín-Vallejo, 2019). In the same species, Trochet *et al.* (2018) found a slight decrease of the T_{pref} of adult males and females from ~31–32 °C at 500 m elevation to ~29–30 °C at 2000 m elevation during late spring. Irrespective of this possibility, the fact that T_{pref} varied little across a temperature gradient of 6 °C for mean temperature during the activity season suggests that behavioural thermoregulation is optimized at grossly similar body temperatures over the geographical gradient, perhaps because the species is a thermal generalist (Angilletta *et al.*, 2009).

Assuming a strong selection for optimization of thermal performances, behavioural adjustments might explain the absence of geographical variability in T_{pref}. We know that lizards can use microhabitats (e.g. rocks, shaded vegetation or burrows) to maintain an optimal body temperature and buffer the negative effects of cold or hot environmental temperatures on their performance (Gaudenti *et al.*, 2021; Taylor *et al.*, 2021). Hence, macroclimatic conditions, quantified here by average air temperatures, might be less relevant than microclimatic conditions, because common lizards can shift microhabitat selection to maintain an optimal temperature even in the extreme parts of our geographical gradient (Rozen-Rechels *et al.*, 2021). This supposes a great spatial variation in operative temperature to allow efficient thermoregulation even in cold and warm environments (Logan *et al.*, 2019). Ultimately, some components of the ecological niche of the species could also change along the climatic gradients. For example, there might be a shift towards sunny, low-vegetation habitats in high-elevation populations compared with more shaded forest habitats in lowland populations. Complementary studies of the spatial distribution of contemporary populations are required to confirm this hypothesis. However, our thermal cline was substantial, and we found strong morphological differences along the gradient, which were likely to be caused by differences in the duration of the activity season. This suggests that body temperature regulation was constrained, to some extent, by cold climatic conditions early and late in the activity season. Another compensatory mechanism could be the possibility of geographical changes in basking behaviour and effort (Stevenson, 1985): here, animals from cold populations could spend more time basking to maintain an optimal temperature even at the cost of spending less time foraging (Buckley *et al.*, 2015). However, a previous study examined behavioural thermoregulation between lowland and highland populations of *Z. vivipara* and found no compensatory behaviour of this type at high elevation (Gvoždík, 2002). In any case, our results suggest that some aspects of the thermal preferences or the ecological niche did compensate, at least in part, for the strong macroclimatic differences in environmental temperatures. The behavioural mechanisms allowing common lizards to maintain optimal body temperature and performance across gradients of environmental temperatures are important to study in the future, because they can shield organisms from environmental variation and cause evolutionary inertia of physiological traits (i.e. Bogert effect; Muñoz, 2022).

Overall, our study of intraspecific variation of morphology and thermo-hydroregulation traits in *Z. vivipara louislantzi* indicated that macroclimate gradients were the main determinants of geographical variation in morphology but not thermo-hydroregulation traits. Relict populations from the warm rear edge of the range distribution displayed no particular ecophysiological adaptations. Habitat features, such as water access and forest cover, had a stronger influence on evaporative water loss than thermal conditions. We suggest that habitat features should be included in future comparative physiological studies of terrestrial ectotherms in order to gain a better understanding of their role relative to climatic conditions.

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DATA AVAILABILITY

The data underlying this article are available in *Zenodo* at: https://dx.doi.org/10.5281/zenodo.6366222

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:
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Table S1. Environmental data for sampled populations
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