

# Altitudinal variation in egg retention and rates of embryonic development in oviparous *Zootoca vivipara* fits predictions from the cold-climate model on the evolution of viviparity

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## Keywords:

countergradient variation;  
egg retention;  
evolution of viviparity;  
incubation time;  
thermal preferences.

## Abstract

The evolution of reptilian viviparity is favoured, according to the cold-climate hypothesis, at high latitudes or altitudes, where egg retention would entail thermal benefits for embryogenesis because of maternal thermoregulation. According to this hypothesis, and considering that viviparity would have evolved through a gradual increase in the extent of intrauterine egg retention, highland oviparous populations are expected to exhibit more advanced embryo development at oviposition than lowland populations. We tested for possible differences in the level of egg retention, embryo development time and thermal biology of oviparous *Zootoca vivipara* near the extreme altitudinal limits of the species distribution in the north of Spain (mean altitude for lowland populations, 235 m asl.; for highland populations, 1895 m asl.). Altitude influenced neither temperature of active lizards in the field nor temperature selected by lizards in a laboratory thermal gradient, and pregnant females selected lower temperatures in the thermal gradient than did males and nonpregnant females across altitudinal levels. Eggs from highland populations contained embryos more developed at the time of oviposition (Dufaure and Hubert's stages 33–35) than eggs of highland populations (stages 30–34) and partly because of this difference incubation time was shorter for highland embryos. When analysed for clutches from both altitudinal extremes at the same embryonic stage at oviposition (stage 33), again incubation time was shorter for highland populations, indicating genuine countergradient variation in developmental rate. Our results indicate that temperature is an environmental factor affecting the geographical distribution of different levels of egg retention in *Z. vivipara*, as predicted by the cold-climate hypothesis on the evolution of viviparity.

## Introduction

Widely distributed species, which are found in a variety of thermal environments, frequently show variation in life-history characteristics (Adolph & Porter, 1993), such as body size (insects: Huey *et al.*, 2000; birds: Ashton, 2002; mammals: Yom-Tov & Geffen, 2006), reproductive traits (reptiles: Forsman & Shine, 1995;

amphibians: Liao & Lu, 2012) or annual survival rate (reptiles: Adolph & Porter, 1993). A few widespread species differ in the mode of reproduction along their distribution area, some of them even having both oviparous and viviparous populations (e.g. *Salamandra salamandra*, Buckley *et al.*, 2007; *Lerista bougainvillii*, Qualls *et al.*, 1995). Organisms of the same species showing geographical variation in reproductive modes are ideal to study the evolutionary transition from oviparity to viviparity, as oviparous populations are expected to exhibit intermediate characteristics between both modes of reproduction, such as advanced embryonic stages at oviposition and shorter incubation time (Braña *et al.*, 1991; Smith & Shine, 1997; Oufiero

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& Angilletta, 2006; Telemeco *et al.*, 2010). In addition, oviparous and viviparous conspecifics can be compared avoiding misleading interpretations, as they share an immediate common ancestor and therefore present similar genetic, physiological and ecological characteristics. Among squamate reptiles, the group of vertebrates in which viviparity has evolved most frequently (Blackburn, 1999), three species show intraspecific reproductive bimodality: *Lerista bougainvillii* (Qualls *et al.*, 1995), *Saiphos equalis* (Smith & Shine, 1997) and *Zootoca vivipara* (Braña, 1986).

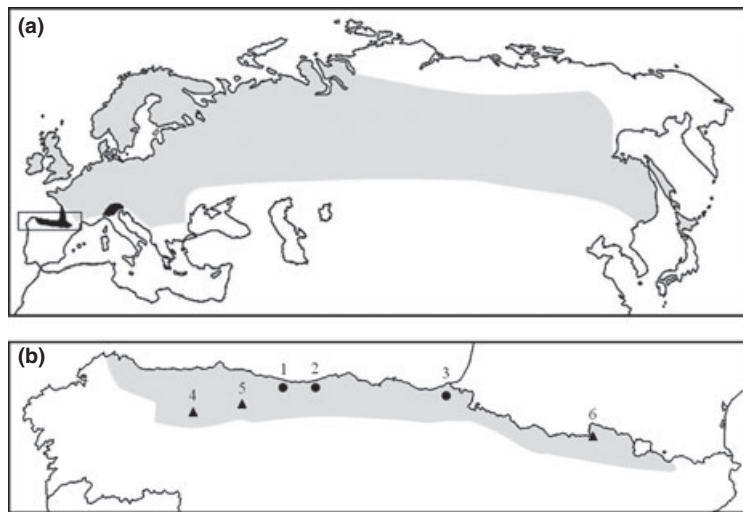
Two main hypotheses try to explain the evolution of viviparity in reptiles, which is generally assumed to occur through selection for progressive increases in the amount of embryonic development in the oviducts (Tinkle & Gibbons, 1977). The maternal manipulation hypothesis (Shine, 1995; Webb *et al.*, 2006) posits that egg retention evolves because females provide eggs with a more stable and warmer thermal environment than the external one, that is, with the optimum thermal environment for embryo development. Evidence on females changing their preferred body temperatures during pregnancy (Shine, 2006), as well as on incubation temperatures similar to those preferred by non-pregnant females having detrimental effects on embryonic development supports this hypothesis (Rodríguez-Díaz & Braña, 2011). On the other hand, the cold climate hypothesis (Shine, 1985, 2002), which is not necessarily incompatible with the maternal manipulation hypothesis (Rodríguez-Díaz *et al.*, 2010), posits that egg retention is favoured at high altitudes or latitudes, where environmental temperatures are much lower than female body temperatures. At these sites, egg retention would accelerate embryonic development and would reduce external incubation time, thus reducing the amount of time eggs are exposed to low nest temperatures, with negative effects on offspring survival (Qualls & Andrews, 1999). In support of this hypothesis, viviparous populations of reptiles are frequently found at high latitudes or altitudes (e.g. Qualls & Shine, 1998a). However, this pattern could be explained either because the potential for longer periods of egg retention would have favoured the evolution of viviparity in cool environments or because viviparity, having evolved elsewhere, would have favoured the subsequent invasion of cool environments (Packard *et al.*, 1977).

The aim of this study consists of testing the cold-climate hypothesis, taking oviparous populations of the lacertid lizard *Zootoca vivipara* (Jacquin, 1797) from the Cantabro-Pyrenean region (Northern Spain) as a model organism. This species has the largest known distribution range among lizards in the world (Gasc *et al.*, 1997) and populations of the Cantabro-Pyrenean region represent the south-western limit of the distribution area of the species (Fig. 1a). In this region, *Z. vivipara* is oviparous, whereas it is viviparous in the rest of its dis-

tribution range, and shows a wide altitudinal distribution, being present from areas close to sea level to 2400 m asl., with a relatively discontinuous distribution because of its habitat specificity (peat bogs and wet meadows; Braña & Bea, 2002). Oviparous populations of this species lay eggs containing embryos at more advanced developmental stages than do other sympatric lizards (i.e. exhibit extended egg retention) and show some interpopulation variability at this respect (Braña *et al.*, 1991). These populations represent an advanced situation in the evolutionary transition from common oviparity to viviparity, being considered the ancestral to viviparous populations (Heulin *et al.*, 2002; but see Surget-Groba *et al.*, 2006). If the evolution of viviparity and egg retention is favoured at low environmental temperature, as posited by the cold-climate hypothesis, high-elevation oviparous populations are expected to exhibit more advanced embryo development at oviposition than low-elevation populations. To test this hypothesis, we assessed altitudinal variation in stage of development at oviposition and incubation time between oviparous populations of the lacertid lizard *Z. vivipara* at the elevational extremes of the species' distribution in the Cantabrian region and the Pyrenees. We also determined whether the thermal environments experienced by low- and high-altitude populations were indeed different and whether females from both altitudes modify their thermal preferences during pregnancy. Additionally, thermal preferences of highland and lowland lizards were assessed to identify whether those of high altitudes and therefore cool environments change their thermal preferences, selecting lower body temperatures than lizards of low altitudes, because the temperatures available in the field for their daily activity would be lower, and this fact could modify the optimal temperature for their physiological processes (Mathies & Andrews, 1995; Angilletta, 2001; Oufiero & Angilletta, 2006). Another possibility might be that highland lizards adjust their thermoregulatory behaviour or their physiological processes to compensate for low temperatures (Gvoždík, 2002).

## Materials and methods

We collected adult male and female *Z. vivipara* in June–July 2008, 2009 and 2010 from six locations of the North of Spain (Fig. 1b): three lowland locations, Buelna (Asturias, 220 m asl.;  $n = 108$ ), Irún (Guipúzcoa, 230 m asl.;  $n = 24$ ) and Treceño (Cantabria, 250 m asl.;  $n = 48$ ) and three highland locations, Leitariagos (Asturias, 1670 m asl.;  $n = 33$ ), Las Señales (León, 1780 m asl.;  $n = 122$ ) and Vielha (Central Pyrenees, Lleida, 2230 m asl.;  $n = 27$ ). These populations as others in the south-western limit of the species distribution range are oviparous (Braña, 1986; Heulin *et al.*, 2000; Surget-Groba *et al.*, 2006), whereas the species is viviparous over most of its range (see Fig. 1a). Lizards were caught by hand or



**Fig. 1** (a) Distribution area of *Zootoca vivipara*. Grey area: viviparous populations; black areas: oviparous populations. (b) Map of the North of the Iberian Peninsula showing the Spanish distribution of *Zootoca vivipara* (grey area) and the collection localities (lowland populations, solid circles: 1 = Buelna, 220 m asl.; 2 = Treceño, 250 m asl.; 3 = Irún, 230 m asl.; highland populations, solid triangles: 4 = Leitarragos, 1670 m asl.; 5 = Las Señales, 1780 m asl.; 6 = Vielha, 2230 m asl.).

noose and were transported to the Zoology laboratory in the University of Oviedo and housed in plastic terraria (50 × 40 × 30 cm; length × width × height) provisioned with a layer of wet substrate, shelter items and water *ad libitum*. Mealworms and crickets were provided twice a day. Heating bulbs were placed at one extreme of the terraria and were switched on from 09:00 to 13:30 h and from 15:30 to 19:30 h each day.

Because rates of embryonic development and other developmental characteristics are highly dependent on environmental temperature, we assessed thermal incubation conditions in the field at both altitudinal extremes. We obtained continuous recordings of temperature in two of the locations studied near both altitudinal limits (Buelna, 220 m asl., and Las Señales, 1780 m asl.) from June 2008 to September 2010. Thermal data loggers (Tidbit v2; Onset Computer Corp., Bourne, MA, USA) were placed at 5 cm depth in potential nest sites of *Z. vivipara* in the field, that is, places similar to those where nests of this species had been previously found.

### Body temperatures of adults

To determine possible altitudinal variation in thermal preferences among oviparous populations of *Z. vivipara*, we measured the temperature selected by adult lizards of each of the six populations studied in a laboratory thermal gradient with a heating bulb (60W) situated in one extreme of a terrarium (range of substrate temperatures: 21.6–40.6 °C). We took one measurement of cloacal temperature for each individual with a *Schultheis* quick-reading thermometer (Miller & Weber, Inc., Ridgewood, NY, USA) one hour and a half after

having placed it in the thermal gradient. Refuges, water or food was not provided during tests, so that lizards would choose their preferred body temperature without being influenced by other biological needs. We distinguished between cloacal temperature of males, pregnant females and nonpregnant females, as it has been demonstrated for oviparous *Z. vivipara* that pregnant females select lower temperatures than the other individuals of the same population (Carretero *et al.*, 2005; Rodríguez-Díaz *et al.*, 2010). Preferred body temperatures were compared to body temperatures of active lizards in the field at two locations near the altitudinal extremes, Buelna (220 m asl.) and Las Señales (1780 m asl.), measured with a *Schultheis* thermometer in a maximum of 10 s after capture to avoid changes in lizards' temperature. Temperatures of the substrate and the air (both shaded for measurement; air temperature taken at 10 cm above the ground) were measured in the site where lizards were captured.

### Adult size and reproductive output

Because altitudinal variation in body size has been frequently reported for ectotherms (Ashton & Feldman, 2003; Angilletta *et al.*, 2004a; Iraeta *et al.*, 2006; Pincheira-Donoso *et al.*, 2008), we measured body mass (digital balance, to the nearest 0.0001 g), snout-vent length (SVL) and head width (digital caliper Vogel, Germany, to the nearest 0.01 mm) of adult males and females of each population. Body mass was used to calculate robustness (size-corrected body mass) of adults.

Cages were checked for clutches at least twice a day. Clutches and females were removed from the terraria and weighed. Relative clutch mass (RCM) was

computed by dividing clutch mass by the mass of the female after egg laying.

### Embryonic stage at oviposition and incubation time

One egg of each clutch was dissected for the determination of developmental stage at oviposition according to Dufaure & Hubert (1961); half stages were assigned if embryos had features intermediate between two developmental stages. The remaining eggs within each clutch were weighed and placed individually in plastic containers with distilled water and vermiculite as substrate (proportion 1/1 in mass). Then, they were randomly assigned to one of the three constant incubation temperature treatments (21, 25 and 29 °C). These temperatures, although higher than mean temperatures experienced by eggs in the nests, were in the range of the temperatures attained there. Incubation time from oviposition to hatching was determined for each population at each temperature.

### Hatchling traits

We measured body mass (digital balance, to the nearest 0.0001 g), SVL, head length, abdomen length and tail length (digital caliper Vogel, Germany, to the nearest 0.01 mm) of hatchlings from the three thermal incubation regimes. We quantified the locomotor performance of hatchlings within 2 days of hatching, by chasing them with a paintbrush along a 1-m length racetrack. Hatchlings were maintained at 32 °C, a temperature that optimizes locomotor performance in this species (Van Damme *et al.*, 1991), for 90 min before the trials. Running trials were recorded with a Nikon video camera at 30 frames per second and locomotor performance was assessed by determining the maximum sprint speed (speed in the fastest five consecutive frames), and the number of stops during trials (see Braña & Ji, 2000).

### Statistical analyses

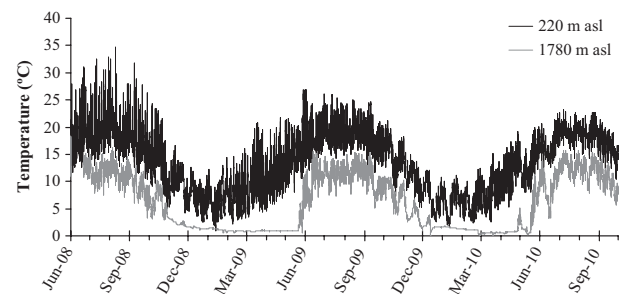
We first assessed whether our data fulfilled the assumptions of normality (Kolmogorov–Smirnov test) and homoscedasticity (Bartlett's test). Clutch size and incubation time were log-transformed to meet these assumptions. Embryo stage at oviposition did not fulfil the above-mentioned conditions even after transformation and is an ordinal variable, so a Kruskal–Wallis test was used to analyse this variable. After the Kruskal–Wallis test, Mann–Whitney *U* tests were performed *a posteriori* with a signification level set at  $\alpha = 0.01$ . Altitude effect on body temperatures of active lizards in the field was analysed by a one-factor (elevation) analysis of variance (ANOVA) and an analysis of covariance (ANCOVA) with air and substrate temperatures as covariates. Nested mixed-model ANOVAS or ANCOVAS were performed to analyse the remaining variables. Population of origin

(nested within the elevation) was included as a random effect in the following analyses: one-factor (elevation) ANOVA for RCM and ANCOVA for clutch size and clutch mass, with female SVL as a covariate; two-factor ANOVA for preferred body temperature of adults (fixed factors: elevation and reproductive condition); and, in the analyses of the phenotype of adult lizards, two-factor (elevation and sex) ANOVA (for SVL) or ANCOVAS (for head width and body mass, with SVL as covariate). As three eggs from the same clutch were distributed among the three incubation treatments, clutch (nested within the population of origin) was included as a random factor along with the population of origin in the analyses of incubation time: two-factor ANOVA (elevation and incubation temperature); and the phenotype of hatchlings: two-factor ANOVAS (for SVL, head length, abdomen length and number of stops) or ANCOVAS (for body mass and tail length with SVL as a covariate, and sprint speed, with SVL and number of stops as covariates). Each of the variables measured is known to affect reproductive success or other traits likely influencing fitness (head length: Gvoždík & Van Damme, 2003; abdomen length: Braña, 1996; tail length: Hofmann & Henle, 2006; sprint speed: Garland *et al.*, 1990), and for this reason, we were interested in assessing each variable separately and we did not carry out multivariate analyses of variance or covariance. Fisher LSD tests were used as tests *a posteriori*. Signification level was set at  $\alpha = 0.05$  for all the analyses.

## Results

### Nest and body temperatures

Recordings of temperatures at potential nests sites in the field revealed that soil temperatures are consistently higher at the low altitude (220 m asl.; mean temperature  $\pm$  SD, during the reproductive period, i.e. from June to September:  $18.04 \pm 2.77$  °C), even occasionally exceeding 30 °C in 2008 (see Fig. 2); on the contrary, maximum temperature hardly reached 15 °C at the



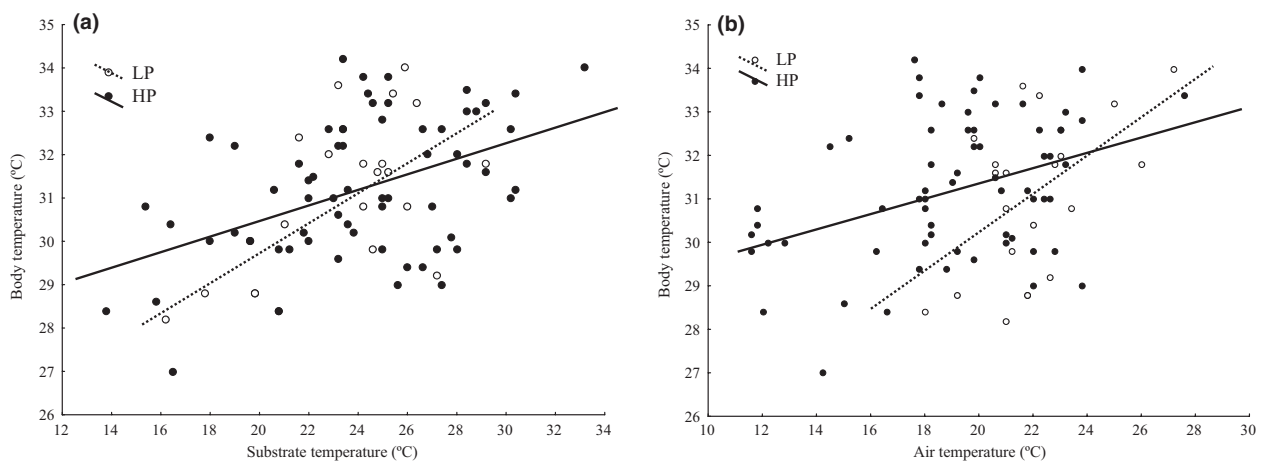
**Fig. 2** Temperatures in potential nests sites of *Zootoca vivipara* located near the altitudinal extremes in the Cantabrian region (Northern Spain), from June 2008 to September 2010.

**Table 1** Body temperatures selected in a laboratory thermal gradient (mean  $\pm$  SD;  $^{\circ}$ C).

	Reproductive condition		
	M	Nongravid F	Gravid F
Lowland populations			
Llanes (220 m asl.)	33.7 $\pm$ 0.8 ( $n$ = 8)	32.1 $\pm$ 2.1 ( $n$ = 9)	31.8 $\pm$ 1.4 ( $n$ = 5)
Irún (230 m asl.)	33.4 $\pm$ 1.6 ( $n$ = 10)	33.3 $\pm$ 0.8 ( $n$ = 5)	31.8 $\pm$ 1.7 ( $n$ = 8)
Treceño (250 m asl.)	33.6 $\pm$ 1.2 ( $n$ = 24)	34.3 $\pm$ 1.2 ( $n$ = 7)	32.7 $\pm$ 1.3 ( $n$ = 16)
Highland populations			
Leitariegos (1670 m asl.)	33.5 $\pm$ 0.7 ( $n$ = 8)	34.0 $\pm$ 1.3 ( $n$ = 22)	31.9 $\pm$ 0.5 ( $n$ = 3)
Las Señales (1780 m asl.)	32.6 $\pm$ 1.8 ( $n$ = 11)	33.3 $\pm$ 1.3 ( $n$ = 15)	33.3 $\pm$ 0.9 ( $n$ = 7)
Vielha (2230 m asl.)	33.2 $\pm$ 2.0 ( $n$ = 7)	32.3 $\pm$ 2.6 ( $n$ = 3)	31.9 $\pm$ 0.8 ( $n$ = 16)

M, males; F, females.

Sample sizes are in brackets.

**Fig. 3** Relationship between field body temperatures of active *Zootoca vivipara* at low- and high-elevation localities (Buelna and Las Señales, respectively) and substrate (a;  $R^2 = 0.37$  for the lowland population;  $R^2 = 0.20$  for the highland population) and air temperatures (b;  $R^2 = 0.27$  for the lowland population;  $R^2 = 0.15$  for the highland population). LP, lowland population; HP, highland population.

high location (1780 m asl.; mean temperature from June to September  $\pm$  SD: 11.09  $\pm$  2.26  $^{\circ}$ C).

We found neither significant altitude effect (ANOVA  $F_{1,174} = 0.065$ ;  $P = 0.81$ ; Table 1) nor between-factor interaction (altitude  $\times$  reproductive condition:  $F_{2,174} = 1.269$ ;  $P = 0.28$ ) when analysing preferred body temperatures of adult lizards. Overall, pregnant females selected lower body temperatures (mean  $\pm$  SD; 32.3  $\pm$  1.2  $^{\circ}$ C) than males and nonpregnant females (33.4  $\pm$  1.5  $^{\circ}$ C; ANOVA  $F_{2,174} = 7.745$ ,  $P = 0.0006$ ). Field body temperatures of lizards active in summer were not affected by altitude (lowland population: 31.0  $\pm$  1.8  $^{\circ}$ C,  $n = 21$ ; highland population: 31.2  $\pm$  1.6  $^{\circ}$ C,  $n = 66$ ; ANOVA:  $F_{1,85} = 0.321$ ;  $P = 0.57$ ; ANCOVA with environment temperatures as covariates:  $F_{1,82} = 0.291$ ;  $P = 0.59$ ; Fig. 3).

### Adult size and reproductive output

Sexual dimorphism was found in all of the three adult traits measured: males are more robust and have

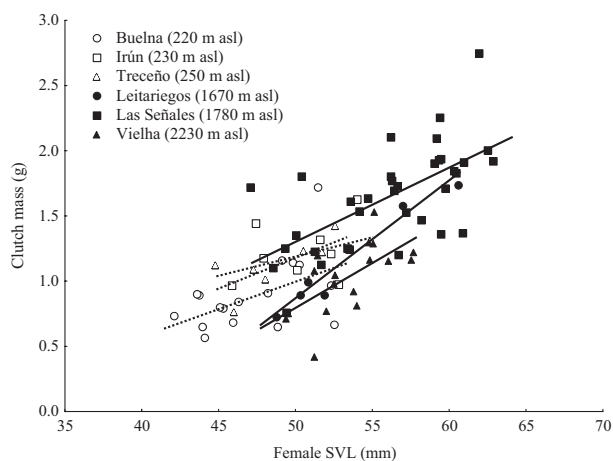
wider head, whereas females have larger SVL. Lizards from low elevations are smaller (SVL) than high-elevation lizards. Altitude did not influence robustness or head width. However, there were elevation  $\times$  sex interactions for head width and robustness: whereas males from low elevations have wider heads and are less robust than males from high elevations, females from low elevations have a similar head width and are more robust than those from high elevations (Table 2).

Clutch size and mass do not differ with altitude when corrected for female SVL (ANCOVAs, for clutch mass:  $F_{1,87} = 0.346$ ,  $P = 0.58$ , Fig. 4; for clutch size:  $F_{1,87} = 0.339$ ,  $P = 0.59$ ). Clutch sizes for the sampled populations were as follows: Buelna, 3–7 ( $n = 17$ ); Irún, 4–7 ( $n = 8$ ); Treceño, 2–7 ( $n = 11$ ); Leitariegos, 3–8 ( $n = 6$ ); Las Señales, 4–9 ( $n = 37$ ); and Vielha, 2–6 ( $n = 17$ ). Relative clutch mass was not influenced by altitude either (ANOVA,  $F_{1,82} = 2.902$ ,  $P = 0.16$ ).

Adult traits	Effects		
	Elevation	Sex	Interaction
Body mass (g)	$F_{1,353} = 0.012$ ; $P = 0.919$	<b><math>F_{1,353} = 224.77</math>; <math>P = 0.000</math></b> <b>M &gt; F</b>	<b><math>F_{1,353} = 33.913</math>;</b> <b><math>P = 0.000</math></b>
SVL (mm)	<b><math>F_{1,354} = 7.438</math>; <math>P = 0.050</math></b> <b>HP &gt; LP</b>	<b><math>F_{1,354} = 49.417</math>; <math>P = 0.000</math></b> <b>M &lt; F</b>	$F_{1,354} = 0.149$ ; $P = 0.700$
Head width (mm)	$F_{1,353} = 0.103$ ; $P = 0.764$	<b><math>F_{1,353} = 352.68</math>; <math>P = 0.000</math></b> <b>M &gt; F</b>	<b><math>F_{1,353} = 5.620</math>;</b> <b><math>P = 0.018</math></b>

HP, highland populations; LP, lowland populations; M, male; F, female.

\*Statistical tests correspond to single effects and between-factor interactions in ANCOVAs (body mass and head width with SVL as covariate) and ANOVA (SVL). Bold face shows statistically significant values.



**Fig. 4** Relationship between clutch mass and female SVL for the six sampled populations: three lowland populations (open symbols; dashed lines) and three highland populations (solid lines and symbols).

### Embryonic development at oviposition and incubation time

Embryo stage at oviposition varied with elevation (Kruskal–Wallis test:  $H(5, n = 94) = 58.089$ ,  $P = 0.000$ ). Mann–Whitney  $U$  tests *a posteriori* revealed that the females from the two highest populations laid eggs with embryos at more advanced developmental stages (Las Señales, and Vielha, stages 33–35) than the females from the other populations (Buelna, Irún, and Treceño, Leitariegos; stages 30.5–34; Fig. 5).

Eggs incubated at higher temperatures hatched sooner (ANOVA,  $F_{2,106} = 13551.000$ ,  $P = 0.000$ ). Furthermore, embryos from high-elevation populations hatched earlier at any of the three incubation treatments (ANOVA,  $F_{1,106} = 11.560$ ,  $P = 0.027$ ; Fig. 6a). In order to assess whether this difference in external incubation time was caused only by the difference in embryo stage at oviposition among lowland and high-

**Table 2** Morphology of *Zootoca vivipara* adults according to the elevation of the population of origin and sex\*.

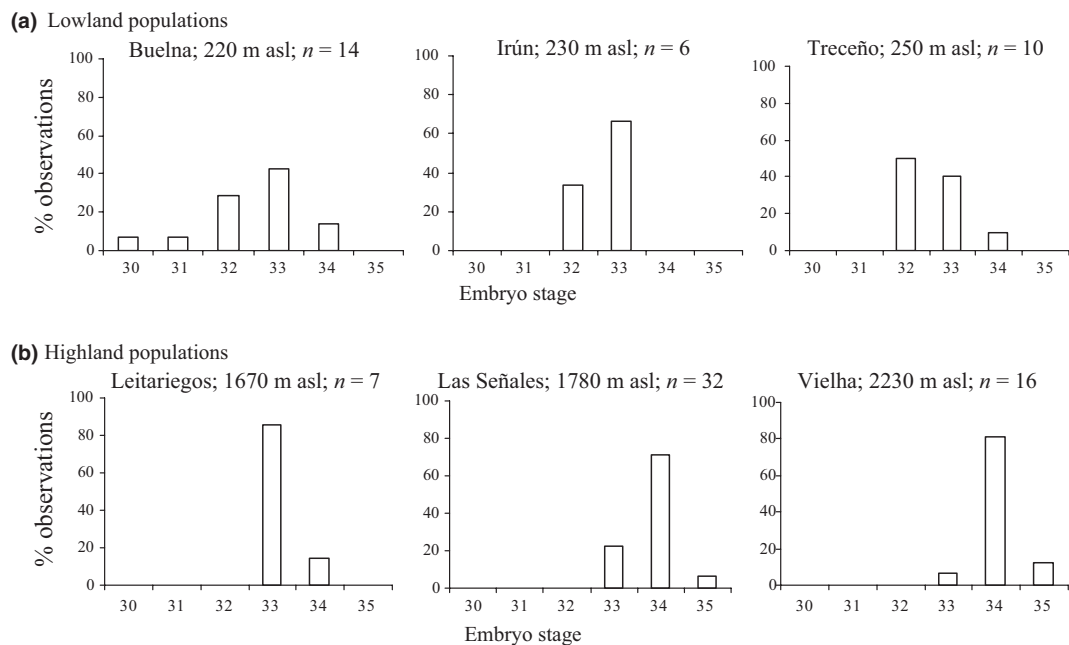
land populations, we analysed incubation time only for clutches with embryos at stage 33 at oviposition because it was the only stage present in both highland and lowland populations. We included in this analysis one lowland population and one highland population having enough clutches at stage 33 to allow the analysis (nested mixed-model ANOVA with the female as a random factor nested within the population of origin, and population and incubation temperature as fixed factors). Again, eggs incubated at higher temperatures hatched sooner ( $F_{2,7} = 3956.5$ ,  $P = 0.000$ ), and incubation time was shorter for embryos of the highland population ( $F_{1,7} = 48.6$ ,  $P = 0.000$ ; Fig. 6b). The altitude  $\times$  incubation temperature interaction effect was not significant ( $F_{1,7} = 0.2$ ,  $P = 0.69$ ).

### Hatchling traits

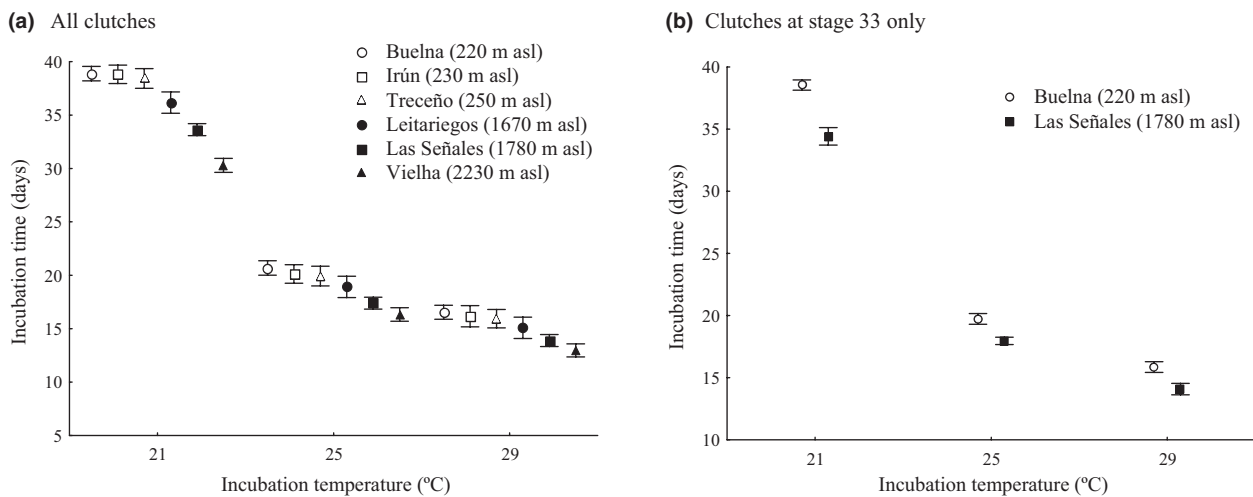
Most hatchling traits differed between populations: SVL,  $F_{4,106} = 5.14$ ;  $P = 0.001$ ; head length,  $F_{4,102} = 3.470$ ,  $P = 0.011$ ; tail length,  $F_{4,105} = 8.888$ ,  $P = 0.000$ ; maximum sprint speed,  $F_{4,100} = 5.957$ ,  $P = 0.000$ ; and number of stops,  $F_{4,103} = 15.803$ ,  $P = 0.000$ ; and some of them between clutches: SVL,  $F_{87,106} = 2.56$ ;  $P = 0.000$ ; body mass,  $F_{87,105} = 6.207$ ,  $P = 0.000$ ; abdomen length,  $F_{87,102} = 1.53$ ;  $P = 0.019$ ; tail length,  $F_{87,105} = 3.038$ ;  $P = 0.000$ . While altitude did not influence any of the hatchling traits measured (Table 3), the thermal regime experienced by embryos affected SVL, tail length and locomotor performance traits: hatchlings from 21 °C were larger (SVL), had shorter tails, ran slower and stopped less often than hatchlings from the other incubation treatments (Table 3).

### Discussion

Oviparous *Zootoca vivipara* lays eggs at very advanced embryonic stages compared to other lacertid lizards (Braña *et al.*, 1991), and our present results show that highland females lay eggs with embryos at more advanced developmental stages (stages 33–35) than



**Fig. 5** Developmental stages at oviposition of embryos of oviparous *Zootoca vivipara* of three lowland (a) and three highland (b) populations studied, according to the stages described by Dufaure & Hubert (1961). Half stages were not depicted: they were assigned to the closer stage when possible. Number of clutches for each population is shown.



**Fig. 6** Incubation time, from oviposition to hatching, at the three constant incubation regimes (21, 25 and 29 °C) for the six sampled populations (a); and for one lowland and one highland population, considering only clutches at stage 33 at oviposition (b). Open and closed symbols represent lowland and highland populations, respectively.

lowland females (30–34), as expected from predictions of the cold-climate hypothesis on the evolution of viviparity. A similar pattern of intraspecific altitudinal variation in the level of egg retention has also been found in other lizards (*Sceloporus scalaris*: Mathies & Andrews, 1995; *Bassiana duperreyi*: Telemeco *et al.*, 2010). According to the cold-climate hypothesis, prolonged egg

retention is most advantageous in cold environments because the mean difference in temperature between eggs in nests and in the female is the greatest at high altitudes. As a consequence, eggs retained by females at high altitudes will be exposed to warmer temperatures on average than they would be in nests and thus egg retention will increase the rate of development and

Hatchling traits	Effects		
	Elevation	Incubation temperature	Interaction
<b>Morphology</b>			
SVL (mm)	$F_{1,106} = 1.980$ ; $P = 0.23$	<b><math>F_{2,106} = 3.710</math>; <math>P = 0.028</math></b> <b>21 &gt; 29</b>	$F_{2,106} = 0.460$ ; $P = 0.63$
Head length (mm)	$F_{1,102} = 0.129$ ; $P = 0.74$	$F_{2,102} = 1.224$ ; $P = 0.30$	$F_{2,102} = 0.092$ ; $P = 0.91$
Abdomen length (mm)	$F_{1,102} = 5.700$ ; $P = 0.07$	$F_{2,102} = 1.100$ ; $P = 0.34$	$F_{2,102} = 0.690$ ; $P = 0.50$
Body mass (g)	$F_{1,105} = 3.574$ ; $P = 0.12$	$F_{2,105} = 1.805$ ; $P = 0.17$	$F_{2,105} = 0.690$ ; $P = 0.50$
Tail length (mm)	$F_{1,105} = 0.002$ ; $P = 0.97$	<b><math>F_{2,105} = 34.893</math>; <math>P = 0.000</math></b> <b>21 &lt; (25, 29)</b>	$F_{2,105} = 0.218$ ; $P = 0.80$
<b>Locomotor performance</b>			
Sprint speed (cm s <sup>-1</sup> )	$F_{1,100} = 2.077$ ; $P = 0.22$	<b><math>F_{2,100} = 8.178</math>; <math>P = 0.0005</math></b> <b>21 &lt; 29</b>	$F_{2,100} = 0.080$ ; $P = 0.92$
Number of stops	$F_{1,103} = 0.000$ ; $P = 0.99$	<b><math>F_{2,103} = 7.224</math>; <math>P = 0.001</math></b> <b>21 &lt; (25, 29)</b>	$F_{2,103} = 0.931$ ; $P = 0.40$

**Table 3** Morphology and locomotor performance of *Zootoca vivipara* hatchlings ( $n = 203$ ) according to the elevation of the population of origin and incubation temperature\*.

\*Statistical tests correspond to single effects and between-factor interactions in ANCOVAs (tail length and hatchling mass with SVL as covariate; sprint speed, with SVL and number of stops as covariates) and ANOVAs (SVL, head length, abdomen length and number of stops). Parentheses below F values show the results revealed by Fisher LSD tests *a posteriori* for the effects of incubation temperatures. Bold face shows statistically significant values.

potentially enhance survival (Andrews, 2000; Shine, 2002).

Congruent with differences in developmental stages at oviposition, embryos from higher populations completed external incubation faster than those from lowland populations at any temperature. The same pattern was found when comparing incubation time of one highland and one lowland Mediterranean species (Monasterio *et al.*, 2011), as well as highland and lowland populations of the same species (e.g. *Sceloporus undulatus*: Oufiero & Angilletta, 2006; Du *et al.*, 2010), presumably due to faster developmental rates of highland embryos, which would be a physiological adaptation to low temperatures (Olsson *et al.*, 1996; Andrews *et al.*, 1999; Angilletta *et al.*, 2004b; Oufiero & Angilletta, 2006). However, most studies that have reported physiological adaptation of embryonic development to cold climates in lizards (Olsson *et al.*, 1996; Qualls & Shine, 1998b; Oufiero & Angilletta, 2006) have not properly determined the developmental stage of embryos at oviposition for each of the studied populations, and therefore cannot appropriately separate the initial development stage of the embryo from genuine differences in developmental rates as the cause of the reported differences in incubation time. The shorter external incubation period for our highland populations might be partly influenced by the embryonic stage at oviposition (see Results) but embryos from high altitudes oviposited at stage 33 still had shorter incubation times and faster developmental rates than embryos from low altitudes oviposited at stage 33. This pattern

of counter-gradient variation (Conover & Schultz, 1995; Conover *et al.*, 2009) entails that high developmental rates compensate for, to some extent at least, low-incubation temperature.

The relative discontinuity between populations throughout the Cantabrian Mountains and the Pyrenees due to the discontinuous habitat distribution suggests that increased egg retention at high elevations might have evolved several times independently, although genetic analyses are needed to confirm this idea. However, considering population clusters resulting from the analyses of geographical variation of the female MPI sex-linked alleles (Guillaume *et al.*, 2000) and mtDNA (Heulin *et al.*, 2011), at least two different origins of prolonged egg retention at high elevations are likely, as the highland population of Vielha (Central Pyrenees) would belong to the eastern sub-group of the SW oviparous clade of *Z. vivipara*, whereas the remaining sampled populations from the Cantabrian region would be included in the western sub-group.

Despite the differences in environmental temperatures experienced by lowland and highland populations in their respective locations of origin, there is no evidence of adaptation of thermal preferendum by adults to local conditions, as selected body temperatures did not differ among elevations. The same has been found for several lizard species (Mathies & Andrews, 1995) including viviparous *Z. vivipara* (Van Damme *et al.*, 1990; Gvoždík & Castilla, 2001). Field body temperatures did not differ between lowland and highland populations, which could point out either behavioural



adaptation of lizards to compensate for cold temperatures at high altitudes (Gvoždík, 2002) or similar thermoregulation opportunities provided by summer thermal environments at both altitudinal extremes, even though activity period is shorter at higher elevations. Field body temperatures were lower than preferred body temperatures both at high- and at low-elevation locations, likely reflecting the difficulty for lizards to thermoregulate accurately in their moist habitat with dense vegetation cover. We have also found for all populations the expected pattern that pregnant females select lower body temperatures than males and non-pregnant females, which has also been reported for other populations of the same species, both viviparous (Van Damme *et al.*, 1986) and oviparous (Carretero *et al.*, 2005). Change in thermal preferences of gravid females toward lower temperatures is probably linked to the maintenance of temperatures suitable for a successful embryonic development, in accordance with the maternal manipulation hypothesis. Therefore, this hypothesis is not incompatible with the cold-climate hypothesis (see Rodríguez-Díaz *et al.*, 2010), as the low temperatures selected by pregnant females would not entail an important increase in the potential incubation time (Rodríguez-Díaz & Braña, 2011).

Adult *Z. vivipara* from high-elevation populations are larger, despite having a shorter activity period in such a cool environment and therefore a shorter growing season. The same pattern has been found in other lizard species, such as *Sceloporus undulatus* (Angilletta *et al.*, 2004a; Sears & Angilletta, 2004) or *Niveoscincus ocellatus* (Wapstra & Swain, 2001), which reach a larger size in cold environments in association with delayed maturity (Adolph & Porter, 1996; Wapstra *et al.*, 2001). In spite that having a large body in cool climates would have a number of benefits, (e.g. higher survival over the hibernation period, Ashton, 2001), most lizards reverse Bergmann's rule. This rule states that species or populations in colder environments tend to have larger body sizes (Blackburn *et al.*, 1999) and the reason why this rule is not valid for most lizards would be that, in cool environments, small lizards gain heat more rapidly than large lizards (Ashton & Feldman, 2003; Pincheira-Donoso *et al.*, 2008). However, different environments at low- and high-elevation sites with respect to food availability or temperature might have influenced the growth of lizards (Niewiarowski & Roosenburg, 1993), and the altitudinal size difference found in *Z. vivipara* may have been the result of delayed maturation in colder environments, instead of genetic divergence.

Our results showed that hatchlings from the lowest thermal treatment (21 °C) were larger (SVL) than those from 25 to 29 °C, which would be advantageous because large individuals have higher overwinter survival (Civantos *et al.*, 1999). However, the lowest thermal incubation treatment had effects on traits relevant to the survival of hatchlings, such as tail length and

locomotor performance (Husak, 2006; Parker & Andrews, 2007). Tail length can influence reproductive success in *Z. vivipara* (Hofmann & Henle, 2006), and locomotor performance has been proved to affect social interactions in several lizard species (*Sceloporus occidentalis*: Garland *et al.*, 1990; *Urosaurus ornatus*: Robson & Miles, 2000). Therefore, according to our results, there is no direct adaptation of highland *Z. vivipara* to enhance hatchling phenotypes at low temperatures, unlike some cold-climate species (e.g. *Nannoscincus maccoyi*, Shine, 1999). Instead, highland *Z. vivipara* seems to avoid low nest temperatures by increasing intrauterine egg retention and developmental rates.

Our finding of different levels of intrauterine egg retention in oviparous *Z. vivipara* depending on the altitude concurs with the view that the evolution of viviparity in reptiles occurs gradually through increasingly developed embryo stages at oviposition (Packard *et al.*, 1977; Qualls *et al.*, 1997). The fact that highland populations attain more advanced embryo stages than lowland populations, together with the geographical distribution of the reproductive modes of *Z. vivipara* (oviparity in several areas at the southernmost limit and viviparity in most of its distribution area), agrees with the expected under the cold-climate hypothesis on the evolution of viviparity in reptiles (Tinkle & Gibbons, 1977; Shine, 1985; Hodges, 2004), which links the evolutionary transition towards viviparity to high altitudes or latitudes, that is, to cold climates.

## Acknowledgments

We are very grateful to Félix González for collection of lizards. Lizards were collected under permit of the environmental authorities of the Junta de Castilla y León, Gobierno del Principado de Asturias, Xunta de Galicia, Conselh Generau d'Aran, Diputación Foral de Gipuzkoa, Gobierno de Cantabria. Funding was provided by the Spanish Ministry of Science (M.E.C.) as a project grant (ref. CGL2007-60187) to Florentino Braña and a fellowship to Tania Rodríguez-Díaz (ref. AP2005-4296) co-financed by the European Social Fund. Adult lizards and hatchlings born in the laboratory were released back into the wild at their places of capture after the experiments were completed.

## References

- Adolph, S.C. & Porter, W.P. 1993. Temperature, activity, and lizard life histories. *Am. Nat.* **142**: 273–295.
- Adolph, S.C. & Porter, W.P. 1996. Growth, seasonality, and lizard life histories: age and size at maturity. *Oikos* **77**: 267–278.
- Andrews, R.M. 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp.): a variant of the cold-climate model. *J. Zool.* **250**: 243–253.

- Andrews, R.M., Mathies, T., Qualls, C.P. & Qualls, F.J. 1999. Rates of embryonic development of *Sceloporus lizards*: do cold climates favor the evolution of rapid development? *Copeia* **1999**: 692–700.
- Angilletta, M.J. 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* **82**: 3044–3056.
- Angilletta, M.J., Niewiarowski, P.H., Dunham, A.E., Leaché, A.D. & Porter, W.P. 2004a. Bergmann's clines in ectotherms: illustrating a life-history perspective with *sceloporine lizards*. *Am. Nat.* **164**: 168–183.
- Angilletta, M.J., Oufiero, C.E. & Sears, M.W. 2004b. Thermal adaptation of maternal and embryonic phenotypes in a geographically widespread ectotherm. *Int. Congr. Ser.* **1275**: 258–266.
- Ashton, K.G. 2001. Body size variation among mainland populations of the western rattlesnake (*Crotalus viridis*). *Evolution* **55**: 2523–2533.
- Ashton, K.G. 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecol. Biogeogr.* **11**: 505–523.
- Ashton, K.G. & Feldman, C.R. 2003. Bergmann's rule in non-avian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57**: 1151–1163.
- Blackburn, D. 1999. Are viviparity and egg-guarding evolutionarily labile in Squamates? *Herpetologica* **55**: 556–573.
- Blackburn, T.M., Gaston, K.J. & Loder, N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Div. Distrib.* **5**: 165–174.
- Braña, F. 1986. Ciclo reproductor y oviparismo de *Lacerta vivipara* en la Cordillera Cantábrica. *Rev. Esp. Herpetol.* **1**: 273–291.
- Braña, F. 1996. Sexual dimorphism in lacertid lizards: male head increase vs. female abdomen increase? *Oikos* **75**: 511–523.
- Braña, F. & Bea, A. 2002. *Lacerta vivipara*. In: *Atlas y libro rojo de los anfibios y reptiles de España* (J.M. Pleguezuelos, R. Márquez & M. Lizana, eds), pp. 236–237. Dirección General de Conservación de la Naturaleza - Asociación Herpetológica Española, Madrid.
- Braña, F. & Ji, X. 2000. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J. Exp. Zool.* **286**: 422–433.
- Braña, F., Bea, A. & Arrayago, M.J. 1991. Egg retention in lacertid lizards: relationships with reproductive ecology and the evolution of viviparity. *Herpetologica* **47**: 218–226.
- Buckley, D., Alcobendas, M., García-París, M. & Wake, M.H. 2007. Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. *Evol. Dev.* **9**: 105–115.
- Carretero, M.A., Roig, J.M. & Llorente, G.A. 2005. Variation in preferred body temperature in an oviparous population of *Lacerta (Zootoca) vivipara*. *Herpetol. J.* **15**: 51–55.
- Civantos, E., Salvador, A. & Veiga, J.P. 1999. Body size and microhabitat affect winter survival of hatchling *Psammotromus algirus* lizards. *Copeia* **1999**: 1112–1117.
- Conover, D.O. & Schultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol. Evol.* **10**: 248–252.
- Conover, D.O., Duffy, T.A. & Hice, L.A. 2009. The covariance between genetic and environmental influences across ecological gradients. Reassessing the evolutionary significance of countergradient and cogradient variation. *Ann. N.Y. Acad. Sci.* **1168**: 100–129.
- Du, W., Warner, D.A., Langkilde, T., Robbins, T. & Shine, R. 2010. The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. *Am. Nat.* **176**: 522–528.
- Dufaure, J.P. & Hubert, J. 1961. Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. *Arch. Anat. Microsc. Morphol. Exp.* **50**: 309–328.
- Forsman, A. & Shine, R. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Funct. Ecol.* **9**: 818–828.
- Garland, T. Jr, Hankins, E. & Huey, R.B. 1990. Locomotor capacity and social dominance in male lizards. *Funct. Ecol.* **4**: 243–250.
- Gasc, J.P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner, P. et al. (eds). 1997. *Atlas of amphibians and reptiles in Europe*. Collection Patrimoines Naturels, 29. Societas Europaea Herpetologica, Muséum National d'Histoire Naturelle and Service du Patrimoine Naturel, Paris.
- Guillaume, C.P., Heulin, B., Arrayago, M.J., Bea, A. & Braña, F. 2000. Refuge areas and suture zones in the Pyrenean and Cantabrian regions: geographic variation of the female MPI sex-linked alleles among oviparous populations of the lizard *Lacerta (Zootoca) vivipara*. *Ecography* **23**: 3–10.
- Gvoždík, L. 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. *Can. J. Zool.* **80**: 479–492.
- Gvoždík, L. & Castilla, A.M. 2001. A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient. *J. Herpetol.* **35**: 486–492.
- Gvoždík, L. & Van Damme, R. 2003. Evolutionary maintenance of sexual dimorphism in head size in the lizard *Zootoca vivipara*: a test of two hypotheses. *J. Zool.* **259**: 7–13.
- Heulin, B., Guillaume, C.P., Vogrin, N., Surget-Groba, Y. & Tadic, Z. 2000. Further evidence of the existence of oviparous populations of *Lacerta (Zootoca) vivipara* in the NW of the Balkan Peninsula. *C. R. Acad. Sci. Paris, Sciences de la vie/Life Sciences* **323**: 462–468.
- Heulin, B., Ghielmi, S., Vogrin, N., Surget-Groba, Y. & Guillaume, C.P. 2002. Variation in eggshell characteristics and in intrauterine egg retention between two oviparous clades of the lizard *Lacerta vivipara*: insight into the oviparity-viviparity continuum in squamates. *J. Morphol.* **252**: 255–262.
- Heulin, B., Surget-Groba, Y., Sinervo, B., Miles, D.B. & Guiller, A. 2011. Dynamics of haplogroup frequencies and survival rates in a contact zone of two mtDNA lineages of the lizard *Lacerta vivipara*. *Ecography* **34**: 436–447.
- Hodges, W.L. 2004. Evolution of viviparity in horned lizards (*Phrynosoma*): testing the cold-climate hypothesis. *J. Evol. Biol.* **17**: 1230–1237.
- Hofmann, S. & Henle, K. 2006. Male reproductive success and intrasexual selection in the common lizard determined by DNA-microsatellites. *J. Herpetol.* **40**: 1–6.
- Huey, R., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**: 308–309.
- Husak, J.F. 2006. Does speed help you survive? A test with collared lizards of different ages. *Funct. Ecol.* **20**: 174–179.

- Iraeta, P., Monasterio, C., Salvador, A. & Díaz, J. 2006. Mediterranean hatchling lizards grow faster at higher altitude: a reciprocal transplant experiment. *Funct. Ecol.* **20**: 865–872.
- Liao, W. & Lu, X. 2012. Adults body size =  $f$  (initial size + growth rate  $\times$  age): explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evol. Ecol.* **26**: 579–590.
- Mathies, T. & Andrews, R.M. 1995. Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris*: implications for the evolution of viviparity. *Oecologia* **104**: 101–111.
- Monasterio, C., Shoo, L.P., Salvador, A., Siliceo, I. & Díaz, J. 2011. Thermal constraints on embryonic development as a proximate cause for elevational range limits in two Mediterranean lacertid lizards. *Ecography* **34**: 1030–1039.
- Niewiarowski, P.H. & Roosenburg, W. 1993. Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. *Ecology* **74**: 1992–2002.
- Olsson, M., Gullberg, A., Shine, R., Madsen, T. & Tegelstrom, H. 1996. Paternal genotype influences incubation period, offspring size, and offspring shape in an oviparous reptile. *Evolution* **50**: 1328–1333.
- Oufiero, C.E. & Angilletta, M.J. 2006. Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution* **60**: 1066–1075.
- Packard, G.C., Tracy, C.R. & Roth, J.J. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class reptilia. *Biol. Rev.* **52**: 71–105.
- Parker, S.L. & Andrews, R.M. 2007. Incubation temperature and phenotypic traits of *Sceloporus undulatus*: implications for the northern limits of distribution. *Oecologia* **151**: 218–231.
- Pincheira-Donoso, D., Hodgson, D.J. & Tregenza, T. 2008. The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizards? *BMC Evol. Biol.* **8**: 68.
- Qualls, C.P. & Andrews, R.M. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biol. J. Linn. Soc.* **67**: 353–376.
- Qualls, C.P. & Shine, R. 1998a. *Lerista bougainvillii*, a case study for the evolution of viviparity in reptiles. *J. Evol. Biol.* **11**: 63–78.
- Qualls, F.J. & Shine, R. 1998b. Geographic variation in lizard phenotypes: importance of the incubation environment. *Biol. J. Linn. Soc.* **64**: 477–491.
- Qualls, C.P., Shine, R., Donnellan, S. & Hutchinson, M. 1995. The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. *J. Zool.* **237**: 13–26.
- Qualls, C.P., Andrews, R.M. & Mathies, T. 1997. The evolution of viviparity and placentation revisited. *J. Theor. Biol.* **185**: 129–135.
- Robson, M.A. & Miles, D.B. 2000. Locomotor performance and dominance in male tree lizards, *Urosaurus ornatus*. *Funct. Ecol.* **14**: 338–344.
- Rodríguez-Díaz, T. & Braña, F. 2011. Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity. *Evol. Biol.* **38**: 352–359.
- Rodríguez-Díaz, T., González, F., Ji, X. & Braña, F. 2010. Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible? *Zoology* **113**: 33–38.
- Sears, M.W. & Angilletta, M.J. 2004. Body size clines in *Sceloporus* lizards: proximate mechanisms and demographic constraints. *Integr. Comp. Biol.* **44**: 433–442.
- Shine, R. 1985. The evolution of viviparity in reptiles: an ecological analysis. In: *Biology of the Reptilia*, Vol. 15 (C. Gans & F. Billett, eds), pp. 605–694. John Wiley and Sons, New York.
- Shine, R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *Am. Nat.* **145**: 809–823.
- Shine, R. 1999. Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. *J. Evol. Biol.* **12**: 918–926.
- Shine, R. 2002. Reconstructing an adaptationist scenario: what selective forces favor the evolution of viviparity in montane reptiles? *Am. Nat.* **160**: 582–593.
- Shine, R. 2006. Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *J. Exp. Zool.* **305**: 524–535.
- Smith, S.A. & Shine, R. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Aust. J. Zool.* **45**: 435–445.
- Surget-Groba, Y., Heulin, B., Guillaume, C.P., Puky, M., Semenov, D., Orlova, V. *et al.* 2006. Multiple origins of viviparity, or reversal from viviparity to oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the evolution of parity. *Biol. J. Linn. Soc.* **87**: 1–11.
- Telemeco, R.S., Radder, R.S., Baird, T.A. & Shine, R. 2010. Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biol. J. Linn. Soc.* **100**: 642–655.
- Tinkle, D.W. & Gibbons, J.W. 1977. The distribution and evolution of viviparity in reptiles. *Misc. Publ. Mus. Zool. Univ. Mich.* **154**: 1–55.
- Van Damme, R., Bauwens, D. & Verheyen, R.F. 1986. Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *J. Therm. Biol.* **11**: 219–222.
- Van Damme, R., Bauwens, D. & Verheyen, R.F. 1990. Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos* **57**: 61–67.
- Van Damme, R., Bauwens, D. & Verheyen, R.F. 1991. The thermal dependence of feeding behaviour, food consumption and gut-passage time in the lizard *Lacerta vivipara* Jacquin. *Funct. Ecol.* **5**: 507–517.
- Wapstra, E. & Swain, R. 2001. Geographic and annual variation in life-history traits in a temperate zone Australian skink. *J. Herpetol.* **35**: 194–203.
- Wapstra, E., Swain, R. & O'Reilly, J.M. 2001. Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **2001**: 646–655.
- Webb, J.K., Shine, R. & Christian, K.A. 2006. The adaptive significance of reptilian viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution* **60**: 115–122.
- Yom-Tov, Y. & Geffen, E. 2006. Geographic variation in body size: the effects of ambient temperature and precipitation. *Oecologia* **148**: 213–218.

Received 28 March 2012; revised 23 May 2012; accepted 24 June 2012