



# Thermal constraints on embryonic development as a proximate cause for elevational range limits in two Mediterranean lacertid lizards

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Local adaptation and range restrictions in alpine environments are central topics in biogeographic research with important implications for predicting impacts of global climate change on organisms. Temperature is strongly coupled to elevation and greatly affects life history traits of oviparous reptiles in mountain environments. Thus, species may encounter barriers for expanding their ranges if they are unable to adapt to the changing thermal conditions encountered along elevational gradients. We sought to determine whether thermal requirements for embryonic development provide a plausible explanation for elevational range limits of two species of lacertid lizards that have complementary elevational ranges in a Mediterranean mountain range (*Psammodromus algirus* is found at elevations below 1600 m and *Iberolacerta cyreni* is found at elevations above 1600 m). We combined experimental incubation of eggs in the laboratory with modelled estimates of nest temperature in the field. In both species, increasing temperature accelerated development and produced earlier hatching dates. The species associated with warmer environments (*P. algirus*) experienced an excessive hatching delay under the lowest incubation temperature. Moreover, newborns from eggs incubated at low temperatures showed poor body condition and very slow rates of postnatal growth. In contrast, eggs of the strictly alpine species *I. cyreni* exhibited shorter incubation periods than *P. algirus* that allowed hatching before the end of the active season even under low incubation temperatures. This was countered by lower reproductive success at higher temperatures, due to lower hatching rates and higher incidence of abnormal phenotypes. Elevational range limits of both species coincided well with threshold temperatures for deleterious effects on embryonic development. We suggest that incubation temperature is a major ecophysiological factor determining the elevational range limits of these oviparous lizards with predictable consequences for mountain distributions under future warmer climates.

Geographic range boundaries of species are governed by environmental conditions and extinction–dispersal dynamics (Brown et al. 1996). Determining whether expansion beyond range boundaries is impeded by limits to physiological tolerances has important implications for predicting the response of species to ecological changes generated by climate change (Lee et al. 2009). Elevational gradients offer unique opportunities to deduce mechanisms responsible for geographic range limits as they encompass clinal variation in ecological conditions (i.e. temperature, precipitation, vegetation type) to which organisms have to adjust in order to persist.

Ectotherms are suitable models to explore local adaptation and range restrictions in alpine environments because of their dependence on environmental temperature (Blanckenhorn 1998, Bird and Hodkinson 2005). In particular, lizards have often been used to assess ecophysiological and behavioural responses to elevation (Van Damme et al. 1989, Iraeta et al. 2006). Some species have

been found to prosper exclusively at high elevations, such as alpine habitats, despite the challenges that cool environments pose for lizards (Carranza et al. 2004). Specialisation is thought to be a consequence of evolutionary adaptation in life histories and/or physiological traits in response to past episodes of geographical isolation driven by historical fluctuations in climate (Avisé et al. 1998). Therefore, if adaptations that have allowed long-term persistence of species in mountains involve traits that are crucial for survivorship in cold environments, they can also affect current distributional limits.

Temperature is one of the major environmental factors that varies with elevation and greatly affects life history traits of oviparous lizards. Cool environments found in mountains shorten the active season available for lizards, which places specific demands on reproductive strategies (Iraeta et al. 2006, Warner and Shine 2007). Low temperatures prolong, and may even prevent, the successful incubation of eggs (Shine 2002) and can limit foraging time prior to

hibernation. In response, lizards may fine-tune hatching dates to enhance the performance and survival of juveniles (Braña and Ji 2000, Shine and Olsson 2003). Incubation temperature can also directly affect egg development. Low temperatures can result in hatchlings with reduced performance and fitness (Qualls and Andrews 1999) and temperatures that are too high can be detrimental for development (Braña and Ji 2000, Ji et al. 2002, Andrews 2008). Thus, embryonic development must be optimized in different species according to the environments to which they are exposed.

Mechanistic models that link physiology of species and the environmental factors limiting distributions provide a valuable tool to generate more realistic assessments of the likely response of species to contemporary climate change (Kearney and Porter 2004, Buckley 2008). These assessments can then be used to develop management strategies that minimise the impacts of climate change (Mitchell et al. 2008). Few studies have incorporated thermal requirements of eggs and embryos into mechanistic models. This is despite recognition that climatic effects on egg development may be equally important as effects on adult stages in determining range limits (Lourdais et al. 2004, Parker and Andrews 2007). Empirical data that link incubation temperature with reproductive success are needed to test specific hypotheses about the role of reproductive physiology of lizards in shaping their range limits. Experimental approaches can be very useful for quantifying physiological sensitivity to incubation temperature (Braña and Ji 2000, Andrews 2008), but the results are best interpreted in the context of environmental exposure directly measured in the field (Williams et al. 2008).

Our goal was to determine whether thermal requirements of embryonic development provide a plausible explanation for distribution limits of two species of lacertid lizards that have mutually exclusive, almost complementary elevational ranges in the Sistema Central Mountain Range, Spain. More specifically, we addressed the following questions: 1) does incubation temperature affect lizard reproductive success through its effects on egg development (i.e. by affecting hatching success or causing abnormal hatchling phenotypes) and/or by modifying hatching dates and growth rates of juveniles; 2) if affirmative, do our study species respond similarly to variations in incubation temperature; and 3) to what extent do elevational ranges of lizards coincide with limits predicted from incubation experiments and actual temperature variation in probable nesting sites in the field?

## Materials and methods

### Study species and study area

The two species examined in this study are distantly related (Arnold et al. 2007), heliothermic, actively foraging, diurnal lacertid lizards found in the Sistema Central Mountain Range. The species have different elevational ranges and are associated with different habitats, although in the study area their ranges are separated by a short distance (ca 5 km). The rock lizard *Iberolacerta cyreni* (mean adult snout vent length [SVL] = 68.1 mm males, 70.2 mm females; range =

65–80 mm) is an alpine species endemic to the Sistema Central, and it is only found above 1600 m, preferring rocky outcrops and mixed rock-shrub formations (Monasterio et al. 2010a). The large psammodromus *Psammodromus algirus* (mean adult SVL = 75 mm males, 75.9 mm females; range = 65–90 mm) inhabits shrub and forested habitats throughout the entire Iberian Peninsula, south-eastern coastal region of France and north-west Africa (Díaz and Carrascal 1991). This species is linked to warmer habitats and is absent from the highest elevations, reaching its upper elevational limit in the Sistema Central at ca 1600 m.

Field work was conducted in the Sierra de Guadarrama, which experiences cold wet winters and short dry summers. The mountain base (1200–1700 m a.s.l.) is covered with deciduous Pyrenean oak *Quercus pyrenaica* forests, which is progressively replaced by Scots pine *Pinus sylvestris* forest at higher elevations. Forest occurs from 1500 to 2100 m a.s.l., gradually becoming less dense at higher elevations until it is dominated by a mosaic of dense mixed-shrub formations of perennial *Juniperus communis* and *Cytisus oromediterraneus* interspersed with small meadows of *Festuca* and other grasses. Alpine areas above the tree line (1700–2300 m a.s.l.) are also characterized by extensive patches of large granite rocks and scree interspersed among shrub formations.

### Collection and husbandry of adults, eggs and juveniles

Between May and June 2008, we noosed 40 adult lizards (*P. algirus*: 15 females and 5 males; *I. cyreni*: 14 females and 6 males) that were transported to the lab (Dept of Zoology, Univ. Complutense de Madrid) on the day of capture. Lizards were housed in white opaque wall terraria (40 × 60 × 30 cm; 2 females per cage). A single male was placed in each of a subset of cages where we could not confirm the presence of oviductal eggs in females at the time of capture). Cages were covered by a green net (0.5-cm mesh) that prevented escape. The mesh provided a shrubby-like shelter while still allowing daylight to enter the cages. Terraria were filled with moistened earth averaging 10 cm in depth, covered by the appropriate vegetation type according to the species inhabiting it. A 60 W lamp suspended over one end of the cage created a photothermal gradient (ca 25–50°C) allowing thermoregulation within the preferred temperature range (Díaz and Cabezas-Díaz 2004). UV light was also provided. An earthenware tile (ca 10 × 15 cm) and a thin section of fallen wood provided additional shade and shelter. Lizards were fed crickets *Acheta domesticus* and mealworms *Tenebrio molitor*, dusted with a commercial vitamin and calcium supplement delivered according to manufacturer's recommendations. All cages were watered ad libitum.

Gravid females were monitored daily to detect changes in body shape or body mass that might indicate egg-laying. When it was clear that a female had laid, eggs were located, removed from the cage, counted, weighed and individually placed in 150 ml plastic cups filled with ca 35 g of moistened vermiculite (10 g vermiculite: 8 g water, which is equivalent to ca – 200 kPa; Tracy 1980). Eggs were

completely covered by the vermiculite, and the jars were closed with a tightly fitting screw top to minimize evaporative water loss. Eggs of the same clutch were distributed evenly over three incubators (MMM Friocell, Germany) set at constant temperatures of 24, 28 and 32°C. Searches for new hatchlings were conducted daily toward the end of the incubation period. Hatchlings were weighed, measured (snout-vent length, SVL), and individually marked by toe clipping. The incubation period (in days) was calculated as the time elapsed between egg deposition and hatching. After females laid their eggs, all lizards were released at their site of capture.

We reared newborns ( $n = 104$ ) within individual plastic terraria (265 mm length  $\times$  162 mm width  $\times$  150 mm height) that offered rock and sand substrates. A 60 W lamp 25 cm above the rock acted as a heat source allowing lizards to bask and UV light was also provided. Juveniles were fed and watered as per adults. To monitor growth of juveniles, we measured (to the nearest mm) and weighed (to the nearest mg) all lizards every four days for a total of 28 d. Size and mass specific growth of juveniles in their first 4 weeks of life was estimated using the formula

growth rate =  $\ln(S \text{ at day } 28 / S \text{ at hatching}) / 28$

where  $S$  is the SVL or weight value. These estimates reflect the proportionate increase in size or mass on a per-day basis (Sinervo 1990). At the completion of the experiment all juveniles were released at their mother's site of capture.

### Presence/absence data

We conducted intensive field sampling of the two species throughout the Sierra de Guadarrama. Standardised surveys were conducted between 2006 and 2009 on sunny days during the lizards' active season (i.e. May to September). We recorded all lizards observed along 200 m transects. We supplemented census data with capture records from other studies we carried out in the Sierra de Gredos, which is also part of the Sistema Central. Additionally, we used presence data of the two species in  $1 \times 1$  km UTM grid cells from the 'Amphibian and reptile database' provided by the Asociación Herpetológica Española for the whole Sistema Central Mountain Range.

### Incubation temperatures available in the field and temperature surfaces

Our analysis required incubation temperature to be estimated for an extensive set of geographic locations in the Sierra de Guadarrama where lizards have been found to be present. This was achieved by interpolating point estimates of soil temperature directly quantified in the field. We sampled representative habitats and environments spread throughout the mountain range between 1200 and 2300 m a.s.l. Sampling covered an area of ca 200 km<sup>2</sup> encompassing all the major alpine and subalpine habitat types in the region. Sampled habitats included rock outcrops with mixed-shrub formations, transition and pure pine forests, and oak forests. Our sampling design incorporated different combinations of elevation, cardinal orientation, slope, and habitat structure.

Unfortunately, information on nesting location is lacking for these species. Therefore, at each location, we quantified soil temperature for a range of plausible nesting microhabitats. Thermochron Ibutton temperature loggers ( $n = 119$ ) were placed at 5 cm depth in soil under three different levels of exposure: bare ground, below rocks with a diameter of ca 30 cm, and in the centre of shrubs with a diameter of 80–100 cm. Bare and rock exposures were located in open areas. Temperature was recorded every hour ( $n = 214\,848$  records across all sites) during the breeding season when eggs were incubated in the wild (late June–early September) and used to estimate the average ground temperature at each location and exposure level (20 June–15 September; average duration of data collection for all loggers was  $79 \text{ d} \pm 1 \text{ d}$ ). In total, we obtained 32 estimates of temperature for bare ground, 39 for under rock, and 37 for under shrub ( $n = 108$  loggers).

Since the final objective was to estimate average daily soil temperature across the breeding season at locations sampled for lizards (i.e. where direct measurements of soil temperature were not always available), we generated continuous temperature surfaces. This was achieved using field estimates of temperature from loggers previously described and spatial information on independent variables that can potentially serve to modify local temperatures. To predict average daily soil temperatures we derived separate linear regression models for each exposure level, using the formula

$$\text{Temperature}_{\text{soil}} = a + bE + cS + dC$$

where  $E$  is elevation,  $S$  is solar radiation and  $C$  is canopy cover. For a given location, soil temperature values were obtained from the logger measurements, and data on each of the independent variables were extracted from GIS layers as follows: elevation – elevation was generated from a 90 m resolution digital elevation model (STRM 90m DEM; available from CGIAR-CSI, Jarvis et al. 2008). Solar radiation – solar irradiation ( $\text{Wh m}^{-2} \text{ d}^{-1}$ ) under clear sky conditions was estimated across the region for the middle day of June, July, August and September using the 90 m resolution DEM and the analysis package *r.sun* (Neteler and Mitasova 2004) in GRASS GIS. Solar irradiation was derived from the sum of all three components of global radiation (beam, diffuse and reflected) calculated at half hourly time steps. The command *r.sun* specifically included the shadowing effect of topography which has the potential to ameliorate exposure to solar radiation in mountainous regions. Monthly estimates of solar radiation were averaged to yield a single estimate of exposure for the time period when eggs are incubated in the wild. Canopy cover – canopy cover was derived from the digitalized vegetation map of Spain (Mapa Forestal de España 1:200 000, provided by the Ministerio de Medio Ambiente y Medio Rural y Marino), that quantified variation in the percentage of tree cover in the study area.

Once multiple regression models were obtained, they were extrapolated to the whole study area to generate continuous temperature surfaces for each level of exposure. Data manipulation and regression analyses were performed in ArcGIS and R ([www.R-project.org](http://www.R-project.org)).

We then overlaid occupied locations for both lizard species on each temperature surface and extracted the predicted soil temperatures at these locations. The same

process was repeated for a background set of available locations. We used a 'target-group' background that was simply a collation of all presence locations of any lizard species in our data set (i.e. locations known to have been sampled for lizards). The 'target-group' approach is preferred to a random background because it is better able to cope with spatial bias in sampling effort (Phillips et al. 2009).

## Results

### Hatching success

We incubated a total of 138 eggs of the two species at three temperatures (Fig. 1). A log-linear analysis of frequencies, with hatching success as the response categorical variable, and species and temperature as the design factors, indicated that the effect of temperature on hatching success differed between the two species ( $\chi^2_2 = 6.11$ ,  $DF = 2$ ,  $p = 0.047$ ). Hatching success of *I. cyreni* was lower at the highest experimental temperature (Fig. 1; lower [24+28°C] vs higher [32°C] temperatures:  $\chi^2_1 = 5.25$ ,  $p = 0.022$ ). Temperature had no effect on hatching success in *P. algirus* ( $\chi^2_1 = 1.75$ ,  $p = 0.186$ ).

### Maternal investment

Species differed in mean egg mass (ANCOVA with SVL as the covariate; species effect:  $F_{1,19} = 16.66$ ,  $p < 0.001$ ) with *I. cyreni* laying heavier eggs (mean egg mass  $\pm 1$  SE =  $0.46 \pm 0.02$  g) than *P. algirus* ( $0.36 \pm 0.02$  g). Clutch size was slightly larger for *P. algirus* ( $6.66 \pm 0.41$  eggs) than for *I. cyreni* ( $6.60 \pm 0.45$  eggs; ANCOVA with SVL as the covariate; species effect:  $F_{1,19} = 4.492$ ,  $p = 0.047$ ). *Iberolacerta cyreni* laid more and larger eggs than *P. algirus*, and its overall reproductive investment (i.e. clutch mass:  $3.04 \pm 0.16$  g or 36.5% of female body mass) was markedly higher than that of *P. algirus* ( $2.40 \pm 0.14$  g or 24.4% of female

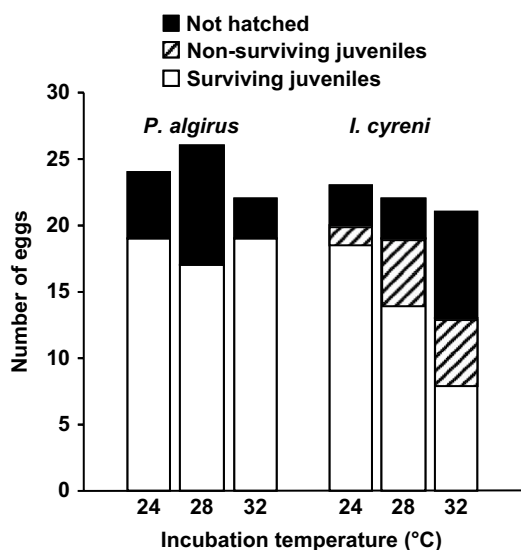


Figure 1. Hatching success of *I. cyreni* and *P. algirus* at different incubation temperatures.

body mass; ANCOVA with SVL as the covariate; species effect:  $F_{1,19} = 28.95$ ,  $p < 0.0001$ ).

### Incubation period

Incubation time differed greatly among species and temperatures (two-way ANOVA of data shown in Fig. 2: temperature:  $F_{2, 101} = 7853.54$ ; species:  $F_{1, 101} = 22684.30$ ; species  $\times$  temperature:  $F_{2, 101} = 1202.01$ ; all  $p$ 's  $< 0.001$ ). Lower temperatures resulted in longer incubation periods, with eggs reared at 24°C taking 1.79 times longer to incubate than those reared at 32°C, and incubation times were 54% shorter for *I. cyreni* than for *P. algirus*. The significant interaction between species and temperature indicated that the intensity of the temperature effect on incubation times differed between both species (Fig. 2); *P. algirus* was more strongly affected by changes in incubation temperature than *I. cyreni*.

Species also differed in their laying dates (ANOVA: species:  $F_{1, 151} = 10503.41$ ,  $p < 0.001$ ; mean laying dates were 24 June and 10 July for *P. algirus* and *I. cyreni*, respectively). Hatching date differed between species and temperatures (two-way ANOVA of data shown in Fig. 3: temperature:  $F_{2, 101} = 7946.46$ ,  $p < 0.001$ ; species:  $F_{1, 101} = 4816.63$ ,  $p < 0.001$ ; species  $\times$  temperature:  $F_{2, 101} = 1131.99$ ,  $p < 0.001$ ). Higher incubation temperatures resulted in earlier hatching dates (mean hatching dates were 7 and 16 August, and 5 September for 32°C, 28°C and 24°C, respectively) and *I. cyreni* hatched earlier (13 August), despite having a later mean laying date than *P. algirus* (26 August). Again, the significant interaction showed that the effect of temperature on laying date was more pronounced for *P. algirus* (mean difference of 40 d between 24°C and 32°C) than for *I. cyreni* (mean difference of 18 d between 24°C and 32°C).

### Viability of hatchlings

Nine *I. cyreni* neonates were born with abnormalities characterized by immobility of one or two of the forelimbs

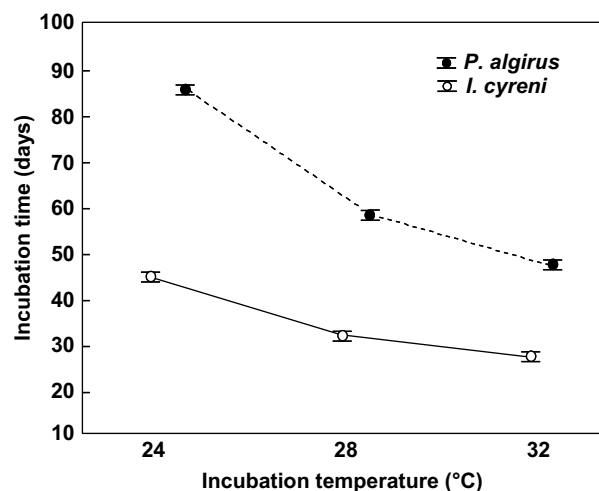


Figure 2. Incubation period of *I. cyreni* and *P. algirus* at different temperatures.

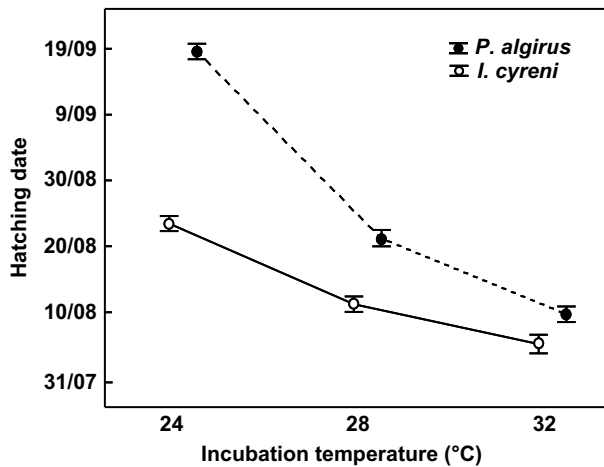


Figure 3. Hatching dates of *I. cyreni* and *P. algirus* at different incubation temperatures.

or an inability to coordinate head movements that hindered feeding and ultimately resulted in mortality. Severity of abnormalities varied among individuals; in some cases, abnormalities were only detected when we observed difficulty feeding of some juveniles. Only one affected lizard (with an immobile forelimb) survived throughout the experiment. The remainder died before the end of the experiment and were classified as non viable juveniles. Unaffected juveniles displayed a normal morphology and feeding behaviour and stayed healthy throughout the experiment. Abnormalities were observed in five of the ten *I. cyreni* clutches. Visual inspection of data suggested a negative effect of incubation temperature on offspring viability (Fig. 1). We estimated for each temperature treatment the proportion of viable juveniles out of the total amount of incubated eggs. The significant result ( $\chi^2 = 7.544$ ,  $p = 0.023$ ) confirmed that increased incubation temperatures had a negative effect on juvenile survival in this species (78, 63, and 38% viable juveniles at 24, 28, and 32°C, respectively).

### Size, body condition and growth rates of juveniles

Body size (SVL) at hatching was larger for *I. cyreni* than for *P. algirus*. Increased incubation temperature produced smaller juveniles in both species (Table 1 and Fig. 4). Body condition at hatching (residuals of log-body mass on log-SVL) did not differ between species, and the main effect of temperature on hatchling body condition was marginally

non-significant. However, the interaction between species and temperature was significant (Table 1). Body condition of *I. cyreni* neonates decreased considerably at higher incubation temperatures. The opposite trend was apparent for *P. algirus* (Fig. 4).

At the end of the experiment (i.e. at an age of 28 d), body condition differed between species and incubation temperatures (Table 1). In both species, juveniles had a better body condition when they came from eggs incubated at higher temperatures, but once again the negative effect of incubation at low temperature was much more pronounced for *P. algirus* than for *I. cyreni* (Fig. 4).

Size and mass specific growth rates also differed between species and incubation temperatures (Table 2 and Fig. 5). Overall, SVL-specific growth rate was highest for *P. algirus* at an intermediate (28°C) incubation temperature; in *I. cyreni*, growth rate was largely independent of incubation temperature. Mass-specific growth rate differed between species and temperatures, being larger for *I. cyreni* than for *P. algirus* and increasing at higher temperatures. In addition, there was a significant species  $\times$  temperature interaction (Table 2); *P. algirus* was much more affected by low temperatures, with hatchlings incubated at 24°C exhibiting very low mass-specific growth rates (Fig. 5).

### Characterization of incubation temperature in the field

Linear regression models explained between 63 and 68% of the variation in soil temperature, depending on the level of microhabitat exposure. Soil temperature on bare ground ( $R^2 = 0.68$ ,  $F_{3,28} = 19.90$ ,  $p < 0.0001$ ) was negatively correlated with elevation ( $\beta = -0.86$ ,  $p < 0.001$ ) and canopy cover ( $\beta = -0.37$ ,  $p < 0.012$ ), but positively associated with solar radiation ( $\beta = 0.35$ ,  $p < 0.003$ ). The same pattern was obtained for soil temperature under rocks ( $R^2 = 0.68$ ,  $F_{3,33} = 19.90$ ,  $p < 0.0001$ ; elevation:  $\beta = -0.86$ ,  $p < 0.0001$ ; canopy cover:  $\beta = -0.30$ ,  $p < 0.025$ ; solar radiation:  $\beta = 0.31$ ,  $p = 0.004$ ). However, the predictive model for soil temperature under shrubs ( $R^2 = 0.63$ ,  $F_{3,32} = 17.87$ ,  $p < 0.0001$ ) included only the effects of elevation ( $\beta = -0.63$ ,  $p < 0.0001$ ) and solar radiation ( $\beta = 0.36$ ,  $p = 0.004$ ), possibly because shrub cover negated the potential effects of canopy cover.

Predicted soil temperatures during the breeding season at occupied locations were consistently lower in microhabitats sheltered by shrubs and higher in rocky or bare ground situations (Table 3). Soil temperatures under different levels of exposure ranged from 20.02 to 27.02°C at locations

Table 1. Two-way ANOVAs for the effects of species (*I. cyreni* vs *P. algirus*), incubation temperature (24, 28, or 32°C), and their interaction, on body size (SVL) and body condition (residuals of log-mass on log-SVL) at hatching and 28 d after hatching.

	Hatchling SVL			Body condition at hatching			Final body condition		
	DF	F	p	DF	F	p	DF	F	p
Species	1	16.27	<0.001	1	0.02	0.888	1	48.21	<0.001
Temperature	2	5.40	0.006	2	2.90	0.060	2	50.19	<0.001
Species $\times$ Temperature	2	0.80	0.452	2	11.59	<0.001	2	19.08	<0.001
Error	100			100			79		

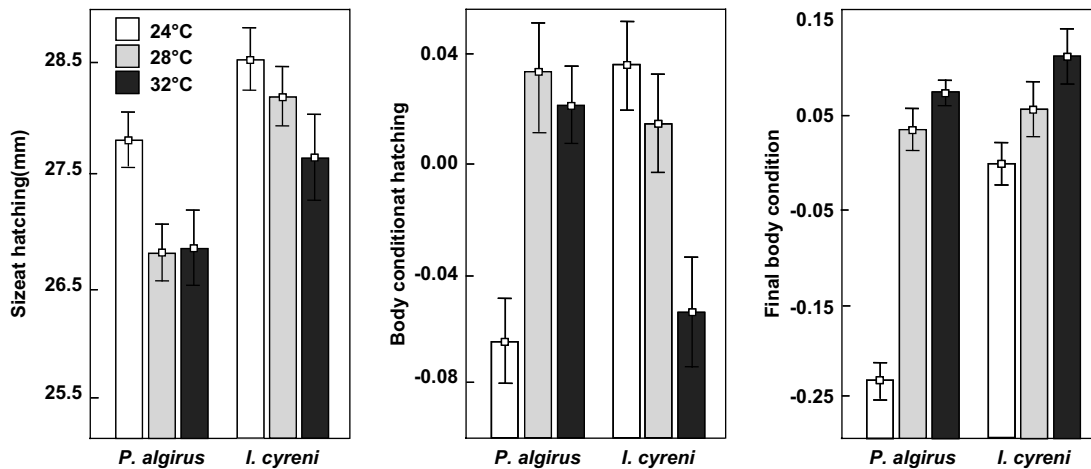


Figure 4. Size (snout-vent length) at hatching, body condition at hatching (residuals of log-body mass on log-SVL), and body condition at four weeks of life of *I. cyreni* and *P. algirus* at different incubation temperatures.

occupied by *I. cyreni* and 27.02 to 35.99°C at locations occupied by *P. algirus*. Overall, however, soil temperatures were strongly biased toward cool temperatures for *I. cyreni* (below 24°C) and the warm temperatures for *P. algirus* (above 24°C except for microhabitats sheltered by shrubs, Table 3, Fig. 6).

## Discussion

Our results support a direct connection between elevational range limits and conditions required for embryonic development in two species of lizards. Both species showed a strong response to incubation temperature with higher temperatures leading to earlier hatching dates. This is consistent with studies elsewhere (Packard and Packard 1988, Van Damme et al. 1992). The species associated with warmer environments (*P. algirus*) experienced an excessive hatching delay under low incubation temperatures that would have prevented successful emergence during the active season. In contrast, the species linked to cold habitats (*I. cyreni*) showed shorter incubation periods at all temperatures, an obviously advantageous trait in an environment where the active season is limited, but exhibited reduced juvenile viability at high temperatures. In the following section, we provide a detailed discussion of the ecophysiological mechanisms that can restrict lizard distribution.

## Life history strategies and incubation temperature dependence: mechanisms of range restriction

### Incubation time and emergence timing: the challenge of alpine environments

It is common knowledge that prolonged incubation under cool conditions can delay hatching, reduce hatching success, and produce low-quality hatchling phenotypes (Braña and Ji 2000, Shine 2002). Our experimental results revealed a strong effect of temperature on incubation time and hatching date in both species. However, the lower elevation species was disproportionately affected. *Psammodromus algirus* incubated under cold regimes were born with a reduced body condition and too late in the active season. This is despite some evidence that this species can adjust life history traits to reduce incubation periods at higher latitudes (Díaz et al. 2007) or altitudes (Iraeta et al. 2006). Low temperatures delayed hatching until mid-September which is considerably later than documented hatching dates for the species in the study area (i.e. second week of August, Iraeta et al. 2006). In addition, juveniles showed very low activity rates and grew at a strikingly slow rate during the first four weeks of their life, which led to a very poor body condition which can compromise their overwinter survival (Civantos and Forsman 2000).

In contrast, even the lowest temperature used in the experiment resulted in adequate hatching dates for *I. cyreni*—the species inhabiting colder environments in the study. Neither high nor low experimental incubation temperatures seemed to limit hatching time for this species. It has been

Table 2. Two-way ANOVAs for the effects of species (*I. cyreni* vs *P. algirus*), incubation temperature (24, 28, or 32°C), and their interaction, on size-specific and mass-specific growth rates of juveniles during their first four weeks of life.

	Size-specific growth rate			Mass-specific growth rate		
	DF	F	p	DF	F	p
Species	1	0.88	0.350	1	30.25	<0.001
Temperature	2	3.58	0.032	2	35.29	<0.001
Species × Temperature	2	4.33	0.016	2	10.72	<0.001
Error	79			79		

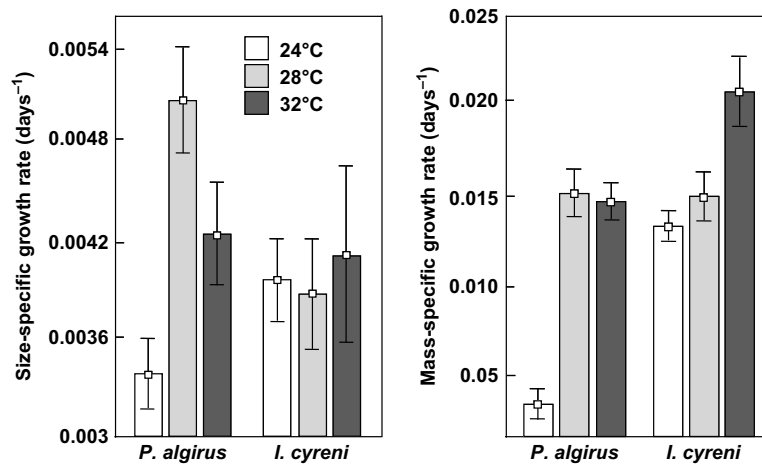


Figure 5. Size and mass specific growth rates of *I. cyreni* and *P. algirus* at different incubation temperatures. See methods for details.

proposed that, because the thermal regimes of cold nest sites are less favourable to embryogenesis than those experienced by oviductal eggs, females could choose to retain their eggs and lay them in a more advanced developmental stage (Qualls and Shine 1998, Braña and Ji 2007). Unfortunately, we currently lack data on development stage at oviposition to assess whether this phenomenon is contributing to the disparity in hatchling dates observed in our study.

**High incubation temperatures and alpine confinement: costs on hatching success and viability**

Increased incubation temperatures reduced hatching success and produced abnormal phenotypes of *I. cyreni* that died before the completion of the experiment, reducing the overall reproductive success at higher temperatures. In reptiles, early events of embryogenesis are involved in tissue differentiation and organogenesis to ensure the production of viable phenotypes, whereas late development is characterized by growth in size and physiological changes (Deeming and Ferguson 1991). Nevertheless, it has also been demonstrated that temperature variation during the external incubation period can affect some morphological features of hatchling lizards (Braña and Ji 2007). This could be the case for *I. cyreni*, in which exposure to high temperatures during the post-laying period of egg development imposed severe costs on the viability of offspring. The lethal effect of high temperatures on embryos has been studied in some species, and the upper threshold seems to be relatively lower in lizards inhabiting cold habitats (as it is

the case for *I. cyreni*) than in lizards occupying warmer areas (Ji et al. 2002, Lu et al. 2006). Although it is known that too high incubation temperatures generate deformities in embryos (Ji et al. 2002), there is a lack of studies documenting temperature-induced phenotypic abnormalities in newborns. Thus, we hope that our results will help draw attention to this potentially interesting thermal effect on the development of reptilian hatchlings.

**Importance of incubation temperature in constraining distribution limits**

The temperature value of 24°C was a critical threshold in this study for the following reasons: 1) it was the experimental temperature under which the reproduction of *P. algirus* was time-constrained due to overextended incubation; and 2) it was the incubation treatment above which hatchlings of *I. cyreni* exhibited abnormal phenotypes. This threshold value, and associated constraints on embryonic development, coincided remarkably well with predicted soil temperatures at potential nesting sites for both species. That is, soil temperatures at sites occupied by *P. algirus* and *I. cyreni* were typically above and below, respectively, the threshold temperature providing strong support for the hypothesis that incubation temperature is acting to constrain range limits. There were, however, some anomalies in the match between embryonic development and local temperature that arose directly from differences in microhabitat exposure. Shrubs provided microhabitats that

Table 3. Predicted breeding season soil temperatures at 5 cm depth under three levels of microhabitat exposure at occupied localities in the Sierra de Guadarrama.

	Exposure	Mean	SE	Minimum	Maximum
All areas (n = 162)	bare	23.45	0.41	9.94	35.99
	rock	22.26	0.38	9.89	33.86
	shrub	18.68	0.29	7.89	27.02
<i>I. cyreni</i> (n = 84)	bare	19.21	0.29	9.94	25.30
	rock	18.29	0.26	9.89	23.84
	shrub	15.72	0.22	7.89	20.02
<i>P. algirus</i> (n = 78)	bare	28.01	0.35	21.90	35.99
	rock	26.53	0.32	21.06	33.86
	shrub	21.86	0.23	17.56	27.02

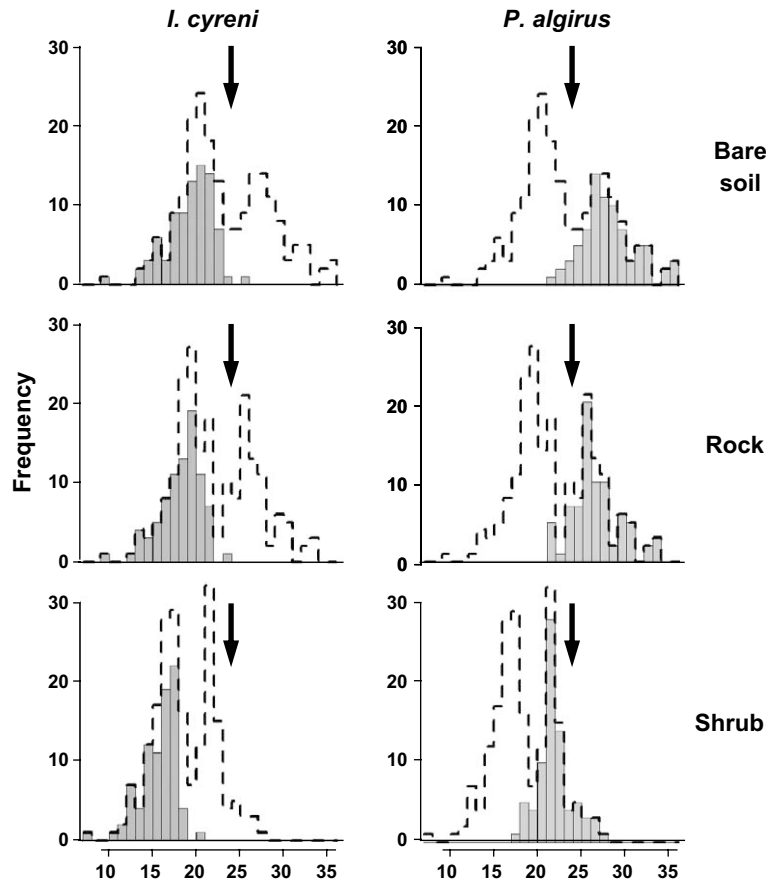


Figure 6. Predicted soil temperatures (x-axis of the figure) at locations occupied by *I. cyreni* and *P. algirus* (shaded bars) relative to a 'target-group' background of available temperatures (unshaded bars) under three levels of microhabitat exposure during the breeding season. Arrows indicate the threshold temperature value of 24°C (see text for an explanation).

were substantially cooler than bare ground or rocks located in open areas. Our predictions suggest that shrubby microhabitats within a large portion of the range of *P. algirus* would be too cold to facilitate complete embryonic development within the activity period. This would suggest that there may be strong selection acting on gravid females to avoid laying eggs in areas densely covered by vegetation. Indeed, nest site selection to ameliorate local exposure to deleterious conditions has been demonstrated in lizards elsewhere (Warner and Shine 2007, Doody 2009). For *I. cyreni*, there is a striking reduction in the frequency of occupied locations as temperatures approach 24°C at potential oviposition sites on bare ground and under rock. However, similar temperatures in shrub microhabitats extend to lower elevations, but are apparently not used by this species. This suggests that there may be some sort of avoidance on the part of *I. cyreni* for laying eggs in this type of microhabitat. Unfortunately, despite several years of intensive investigation, no nests of either species have been located in the field. Thus, it is not yet possible to evaluate microhabitat based nest site avoidance.

A limitation of this study is that temperatures chosen for the laboratory experiment only spanned a subset of the temperatures experienced by lizards in the field. Nevertheless, it is remarkable how well approximate thresholds for physiological development documented in the laboratory matched predicted temperatures within range limits.

The case of *I. cyreni*, the alpine endemic, is especially illustrative. Several potential mechanisms underlying its lower elevational limit have been proposed (Monasterio et al. 2010a), but a proximate cause has not been substantiated. Neither competition with wall lizards (Monasterio et al. 2010b) nor thermal quality of the habitat for adults (Monasterio et al. 2009) provide an adequate explanation for the distribution pattern of *I. cyreni* in this mountain range, although thermal constraints linked with the scarcity of appropriate refuges may partially explain the barrier effect caused by the pine-forest vegetation belt (Monasterio et al. 2009). Temperatures examined in the experiment reported here showed a reduction in overall viability of hatchlings at incubation temperatures equal or higher (at least for bare ground and rock substrates) than those found at the lower elevational range limit of *I. cyreni*. The ecophysiological adaptations to alpine environments, attained after long-term persistence in mountains (Carranza et al. 2004), may therefore prevent these lizards from colonizing lower elevations. On the other hand, *P. algirus*, which is linked to warmer habitats than *I. cyreni*, was generally found above the predicted temperature threshold. Our findings corroborate previous studies in which low temperatures at available nesting sites were found to limit the distribution ranges of other temperate lizards (Hare et al. 2004, Parker and Andrews 2007).



## Implications for global warming

Regional temperatures in Mediterranean mountains are projected to rise considerably over the coming century (Nogués-Bravo et al. 2007). This is expected to have global effects on component ecosystems, with temperature specialists being particularly vulnerable (Ghalambor et al. 2006). We have described a mechanism whereby ground temperature at incubation sites may determine the range limits of two lizard species in the Sierra de Guadarrama. From our results, we predict that contemporary climate warming will result in an upward elevational shift and reduction in range size in *I. cyreni*, which is already listed as endangered in recent IUCN Red Lists because of its limited extent of occurrence and acute fragmentation of its range. Theoretically, rock lizards could compensate for temperature changes by modifying their nesting behaviour (e.g. laying eggs under shrubs), as has been suggested for other species (Doody et al. 2006, Telemeco et al. 2009). However, the species does not appear to take advantage of existing, thermally suitable shrub habitat beyond its current lower elevational range limit. This suggests a low capacity to ameliorate the negative effects of warm temperatures by actively selecting cooler microhabitats. Conversely, we predict a population expansion of the lowland species *P. algirus* towards higher-elevation habitats as warmer temperatures and longer activity periods favour embryonic development.

Information on the phenotypic plasticity of embryonic development in *I. cyreni*, for example populations living at different elevations, would help to distinguish between environmental and population-specific (e.g. genetic) sources of geographical variation (Bronikowski 2000, Iraeta et al. 2006). We recommend assessing the levels of gene flow among central populations, well adapted to alpine conditions, and marginal ones located at lower elevations. This would help clarify the mechanisms preventing local adaptation beyond range limits, and to discern if the mutational input from central into marginal populations is limiting the expansion of locally beneficial alleles, thus hampering adaptation and restricting their potential distribution range (Bridle and Vines 2006).

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