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Influence of Incubation Temperature on Morphology, Locomotor Performance, and Early Growth of Hatchling Wall Lizards (*Podarcis muralis*)

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ABSTRACT Eggs of wall lizards (*Podarcis muralis*) were incubated at three temperatures approaching the upper limit of viability for embryonic development in this species (26, 29, and 32°C) to assess the influence of temperature on various aspects of hatchling phenotype likely affecting fitness. The thermal environment affected size and several morphometric characteristics of hatchling lizards. Hatchlings from eggs incubated at 32°C were smaller (snout-vent length, SVL) than those from 26 and 29°C and had smaller mass residuals (from the regression on SVL) as well as shorter tail, head, and femur relative to SVL. Variation in the level of fluctuating asymmetry in meristic and morphometric traits associated with incubation temperatures was quite high but not clearly consistent with the prediction that environmental stress associated with the highest incubation temperatures might produce the highest level of asymmetry. When tested for locomotor capacity in trials developed at body temperatures of 32 and 35°C, hatchlings from the 32°C incubation treatment exhibited the worst performance in any aspect considered (burst speed, maximal length, and number of stops in the complete run). Repeated measures ANCOVAs (with initial egg mass as covariate) of snout-vent length and mass of lizards at days 0 and 20 revealed significant effects of incubation temperature only for mass, being again the hatchlings from eggs incubated at 32°C those exhibiting the smallest final size. All together, our results evidenced a pervasive effect of thermal regime during incubation (and hence of nest site selection) on hatchling phenotypes. However, incubation temperature does not affect hatchling phenotypes in a continuous way; for most of the analysed traits a critical threshold seems to exist between 29 and 32°C, so that hatchlings incubated at 32°C exhibited major detrimental effects. *J. Exp. Zool. 286:422–433, 2000.*

There is a growing evidence that environmental conditions during embryogenesis can induce phenotypic variation in animals. Temperature is a particularly important factor in determining developmental rates and final size in ectotherms (e.g., Atkinson, '94; Johnston et al., '96) and also has a strong influence on a number of morphological, behavioural, and performance-related traits (e.g., Cossins and Bowler, '87; Packard and Packard, '88; Huey and Berrigan, '96). In reptiles, temperature-dependent sex determination is undoubtedly the most striking phenomenon in this field (Bull, '80; Janzen and Paukstis '91; Lang and Andrews, '94), but many other temperature effects have been demonstrated on traits likely affecting organismal fitness (Webb and Cooper-Preston, '89; Burger, '91, '98; Van Damme et al., '92; Shine and Harlow, '93; Allstead and Lang, '95; Shine, '95; Shine et al., '97a,b). Apart from gross abnormalities or failure of development at extreme temperatures (e.g., Vinegar, '74), there are some other comparatively minor modifications in thermally plastic traits that are likely to be permanent (e.g., sex, vertebrae number, and some morphometric characteristics) or are prone to consolidation or reinforcement (e.g., those linked to hatchling size

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and initial growth), and would therefore have long term effects, thus influencing life-time fitness.

Females of several viviparous or prolonged egg-retaining squamates have been reported to shift selected body temperatures when pregnant (e.g., Beuchat, '88; Andrews and Rose, '94; Mathies and Andrews, '97) to provide optimal thermal environments for developing embryos. In some lacertid lizards pregnant females selected lower body temperatures (*Lacerta vivipara*, Van Damme et al., '86; Heulin, '87; see Braña, '93, and Tosini and Avery, '96, for *Podarcis muralis*), and this suggests that high incubation temperatures could have detrimental effects on hatchling phenotypes. Therefore, we aimed to explore the effect on development of temperatures that are close to the upper critical limit, on the basis of the thermal dependence of both developmental times and hatchling success of *P. muralis* reported by Van Damme et al. ('92). Considering the minimal time-lag between the dates of clutch laying and the emergence of hatchlings observed in the field (Braña, unpublished data), we fixed a lower level at 26°C compatible with fast development rates experienced in natural nests of wall lizards. Similar upper limits have been reported for incubations of *Podarcis hispanica atrata* (Castilla and Swallow, '96) and *Podaris bocagei* (Galán, '94) in the field. Successive temperature levels (29 and 32°C) were fixed to test the effect of temperatures higher than the ones prevailing in natural incubations but still within the range producing high proportions of viable offspring (Van Damme et al., '92; Ji and Braña, 2000a). A number of recent papers on the effects of incubation temperature in reptiles have preferred the simulation of nest thermal and hydric environment rather than applying constant regimes. We have selected three constant temperature regimes mainly because of the multiplicity of possible and realistic combinations when fluctuating conditions are incorporated. In fact, the mean and amplitude of the thermal fluctuations, as well as the water potential, can change daily over several weeks or months through the incubation period, and all of these conditions can experience important variations depending upon the locality, microhabitat, year, and date of egg laying (see, e.g., Overall, '94; Castilla and Swallow, '96; Thompson et al., '96; Shine et al., '97b). Therefore, each nest represents in practice an absolutely particular combination of average temperature, thermal fluctuations, and moisture, among other potentially influencing factors, being a constant temperature just one of these possibilities. In addition, although thermal fluctuations can certainly influence some aspects of the egg development in reptiles, the reported effects were generally of moderate importance (e.g., Packard and Packard, '88; Overall, '94; Shine and Harlow, '96; Andrews et al., '97).

Van Damme et al. ('92) documented the influence of incubation temperature on the duration of the incubation, hatchling size, locomotion, and growth in the wall lizard. In a previous study we analysed the effects of incubation temperature on some energetic aspects of the embryonic development (Ji and Braña, 2000a). Here we examine variation in hatchling phenotypes, considering both morphological and performance-related traits, associated with incubation temperatures, with two main objectives: (1) to evaluate how much morphological variability can generate variation in thermal conditions during incubation and (2) to explore some potential functional links through which phenotypic differences produced by the incubation environment could be translated into differences in the organismal fitness. Morphological evaluation included body axis lengths (snout-vent and tail), robustness (mass residuals), and head and leg measurements. Deviations from bilateral symmetry were tested for meristic (scale counts) and morphometric traits (see Materials and Methods), under the hypothesis that asymmetry (fluctuating asymmetry in particular but also, perhaps, directional asymmetry and antisymmetry; see Graham et al., '93; McKenzie and Yen, '95) could be an indicator of developmental instability negatively correlated with fitness (Palmer and Strobeck, '86; Møller, '97).

Finally, hatchling performance was examined for locomotion and initial growth, two measurements frequently used in evolutionary studies of reptiles because they are both easily measured in the laboratory and ecologically relevant, the later being a necessary condition to allow extrapolation to field conditions (Arnold, '83; Pough, '89). Because all of these traits are assumed to have implications on fitness, results could allow predictions with regard to nest site selection, limits of distribution, and the likely effects of climatic change on the population.

MATERIALS AND METHODS

Eggs were obtained from 53 adult females collected in April 1996 from a lowland population in the neighbourhood of Oviedo, Northern Spain, and maintained in terraria at the Zoology Laboratory of the University of Oviedo. Oviposition (up to three clutches per female) occurred between 22 April and 7 July, paralleling the oviposition cycle in the field. Oviposition usually occurs in our population of *P. muralis* when embryos are at stages 25–29 (according to the development table by Dufaure and Hubert, '61; Braña et al., '91). All the females collected in the field had oviductal eggs and were picked individually from several different plots, so that the male parents for the first clutch were almost certainly all different. Eggs of later clutches were sired in unknown proportion by six males that shared the terraria with the females. Eggs were collected from the oviposition substrate (generally within less than 1 hr after egg laying), individually weighed, and assigned to one of the incubation treatments. Because of the small clutch size of *P. muralis* (3–11 eggs per clutch in our sample; see Ji and Braña, 2000b) and the utilisation of some eggs in a parallel experiment, we were unable to complete a balanced design with respect to family (female parent) assignation among treatments, so that eggs were distributed as equally as possible among treatments. Because of the scattered egg allocation to treatments, the high number of females, and the multiple paternity (even within clutches; authors' unpublished data), we are confident that family effects are unbiased and nonrelevant to the overall results. Egg mass or neonate snout-vent length were used as covariates in several analyses for morphometric and performance-related traits, so that maternal effects operating through egg size were also minimised.

Eggs were incubated at 26, 29, and 32°C (± 0.3°C), placed individually in covered plastic containers (100 ml), and half-buried in a moistened vermiculite substrate. Most eggs for this experiment (73.3%) were incubated at the same moisture level at all temperatures (2 g water/1 g vermiculite; resulting approximately a –12 kPa water potential), but a number of eggs in the temperature treatments of 29 and 32°C were incubated in the proportions 1/1 (–220 kPa) and 3/1 (0 kPa). Containers were weighed daily and, if necessary, distilled water was added to compensate for small evaporative losses and water absorption by the eggs, so that the water potential of the substrate was maintained constant (further details in Ji and Braña, 2000a). Preliminary analyses of variance considering effects of humidity and its interaction with temperature (at 29 and 32°C) revealed that, within the range here considered, moisture was not an important source of variation for those aspects of lizard's phenotype under analysis, and therefore data for different moisture level were pooled within each temperature treatment.

A total of 110 hatchlings were measured (snout-vent length, tail length), weighed, and frozen immediately after hatching. On these hatchlings, mainly utilised to investigate energetic balances through incubation at different temperatures (Ji and Braña, 2000a), we have taken more complete and precise measurements, necessary to examine thermal effects on morphometry and bilateral symmetry. Morphological measurements were: snout-vent length (SVL), mass, tail length, head length and width, humerus length, and femur length. Left–right symmetry was evaluated after measuring both sides for several morphometric (eye diameter, tympanum diameter, distance from the eye to the nasal opening, and fore and hind limbs) and meristic (number of series of ventral plates, upper labial scales, and supraciliary scales) bilateral traits.

One independent sample ($N = 69$) was utilised to evaluate the effect of incubation temperature on locomotor performance and early growth of lizards. To avoid excessive manipulation, only basic measurements were taken on these lizards at hatching (snout-vent length, tail length, mass). We determined running performance of these hatchlings within the two days of hatching and prior to feeding. Because locomotor performance is highly sensitive to changes in body temperature in reptiles (Huey and Kingsolver, '89; Bauwens et al., '95), we developed trials at constant body temperatures of 32 and 35°C, representing respectively an approximate lower threshold for continuous distribution of body temperature of active lizards in the field (see Braña, '91, '93) and the optimal temperature for locomotory performance in *P. muralis* (Bauwens et al., '95; Braña, unpublished data). Body temperature of hatchlings was controlled by placing them in an incubator at the correspondent temperature for at least 30 min prior to testing. Locomotor performance was assessed by chasing down the lizards along a 1.2-m racetrack with one side transparent, which allowed lateral filming with a video camera recording at constant 25 frames · s^{-1}. Each lizard was run three times at each temperature with at least a 15-min resting period between successive trials. Lizards were tested at 32 and 35°C in two successive days. Videotapes were examined for sprint speed in the fastest 20 cm interval (or in the fastest 4 consecutive frames, if no

20 cm of continuous running were available), maximal distance travelled without stopping, and number of stops during the entire trial. Maximal values for sprint and distance, and minimum ones for stop number, were considered as representative of hatchling locomotor performance at the two temperatures.

Post-hatching growth was evaluated in the initial 20-day period. Subsequent to the running trials, we moved hatchlings to large terraria with access to food (small crickets, wingless fruit flies, and small mealworms) and water (supplemented with vitamins and minerals) in excess. Terraria were illuminated with natural (indirect) light, and opportunity for thermoregulation was provided by 100 W light bulbs suspended 20 cm above the floor.

Analyses of variance (for SVL) or covariance (with the SVL as covariate, for all other traits) were used to determine whether incubation temperature affected size, robustness, or morphometry. In addition to the univariate tests, a principal component analysis (varimax rotation) was used to investigate the possible existence of morphological spaces characteristic of hatchlings from different incubation temperatures. Our focus was to describe variation in shape, and consequently we utilised size-corrected values (residuals from the regression on SVL) for all the variables. Differences in fluctuating asymmetry among incubation temperatures were tested using Levene's tests to compare the variances of the signed differences of the measurements from each side, scaled by individual [$(R_i - L_i)/((R_i + L_i)/2)$; index 6, in Palmer and Strobeck, '86]. This index has high discriminatory ability at detecting true differences in fluctuating asymmetry among samples and is relatively insensitive to the existence of directional asymmetry (Palmer and Strobeck, '86). The existence (and possible differences among incubation temperatures) of significant side effects indicative of directional asymmetry were tested with repeated-measures analyses of variance on actual right and left side values of each trait. We carried out repeated-measures ANOVAs (or ANCOVAs, with the appropriate covariate) to analyse all the locomotor performance variables (speed, maximal continuous distance, stop number) as each hatchling was tested successively at body temperatures of 32 and 35°C. Similarly, growth in the first 20 days (both in SVL and mass) was examined for differences among incubation temperatures with repeated-measures ANCOVAs, with log egg mass as the covariate.

RESULTS

Sex and incubation temperature effects on size and morphometry

As preliminary analyses revealed between sex differences in hatchling morphology, we incorporated sex in addition to incubation temperature in two-factor ANOVAs (or ANCOVAs; see Table 1) to examine their effect and possible interaction in explaining variation of several morphometric traits. Females are larger than males, but there are not between sex differences in body mass relative to snout-vent length. The length of the femur did not vary between sexes, whereas tail length, humerus length, head width, and more outstandingly head length, were larger in males when controlled for the effect of differences in SVL (ANCOVAs; Table 1). The effects of incubation temperature on hatchling phenotypes are very general, as only humerus length and head width were noticeably constant among treatments. It is worth noting that for all morphological traits exhibiting significant treatment effects hatchlings incubated at 32°C are smaller (SVL), lighter (mass relative to SVL; Fig. 1), and shorter (for tail, head, and femur lengths relative to SVL) than those incubated at either 26 or 29°C that, in turn, did not differ in any of the examined traits.

A principal component analysis resolved two components (with eigenvalues >1) from six size-free morphometrical variables, accounting for 62% of variation in the original data (Table 2). The first component (34.5% of variance explained) had high positive loading for size-free values of head length and tail length, so high scores in this axis should represent a comparatively elongated shape. The second axis (27.2%) largely represents the length of both the fore and the hind limbs. Hatchlings from different incubation temperatures had highly significant differences in their scores on the first axis [ANOVA, $F_{2,103} = 13.236$, $P << 0.001$; (26, 29) > 32, Scheffé test] but did not differ in their scores on the second axis ($F_{2,103} = 0.485$, $P = 0.617$; Fig. 2).

The independent sample utilised to test locomotor and growth performances ($N = 67$; two hatchlings were excluded from analyses because uncertainty of sex attribution) gave similar results with respect to morphology. Incubation temperature had no significant effect on snout-vent length in this sample ($F_{2,61} = 1.310$, $P = 0.277$), in which females were larger than males (log SVL; $F_{1,61} = 26.934$, $P < 0.0001$; log egg mass as the covariate). On the contrary, incubation temperature had significant effects on hatchling condition [log hatch-

*TABLE 1. Phenotypes of hatchling lizards (*Podarcis muralis*) according to sex and incubation temperature (mean ± sd)*[1]

		Incubation temperature			Effects		
Hatchling trait		26°C	29°C	32°C	Sex F$_{1,102}$	Incubation temperature F$_{2,102}$	Interaction F$_{2,102}$
SVL (mm)	M	25.44 ± 0.52	25.43 ± 0.86	24.42 ± 1.17	14.213*** F > M	7.718*** (26,29)32	1.502 ns
	F	26.01 ± 0.38	25.85 ± 0.65	25.49 ± 1.08			
Mass (g)	M	0.365 ± 0.024	0.369 ± 0.036	0.330 ± 0.026	0.975 ns	4.026* (26,29)32	1.162 ns
	F	0.386 ± 0.029	0.366 ± 0.033	0.345 ± 0.031			
Tail length (mm)	M	38.67 ± 1.57	39.62 ± 3.99	36.69 ± 2.59	9.522** M > F	6.706** (26,29)32	2.207 ns
	F	38.34 ± 2.41	39.37 ± 2.00	35.23 ± 4.07			
Head length (mm)	M	9.65 ± 0.22	9.75 ± 0.31	9.45 ± 0.31	25.936*** M > F	5.511** (26,29)32	3.175*
	F	9.68 ± 0.32	9.53 ± 0.22	9.25 ± 0.22			
Head width (mm)	M	4.22 ± 0.08	4.29 ± 0.17	4.32 ± 0.14	4.050* M > F	2.857 ns	3.925*
	F	4.30 ± 0.19	4.26 ± 0.15	4.25 ± 0.12			
Humerus length (mm)	M	2.34 ± 0.10	2.41 ± 0.21	2.36 ± 0.13	6.897** M > F	0.046 ns	2.823 ns
	F	2.40 ± 0.15	2.29 ± 0.13	2.25 ± 0.20			
Femur length (mm)	M	4.01 ± 0.16	4.06 ± 0.31	3.78 ± 0.17	1.579 ns	5.960** (26,29)32	3.769*
	F	4.19 ± 0.15	3.91 ± 0.23	3.79 ± 0.18			

[1] F ratios correspond to single effects and factor interactions in two-factor ANOVA (for snout–vent length, SVL) or ANCOVAs (with SVL as covariate, for all other traits). Descriptive statistics for hatchling mass are presented as direct values but analyses were carried on log mass with log SVL as covariate. Parentheses below F values for the effects of incubation temperature group together treatments that did not differ as revealed by Scheffé's tests a posteriori.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

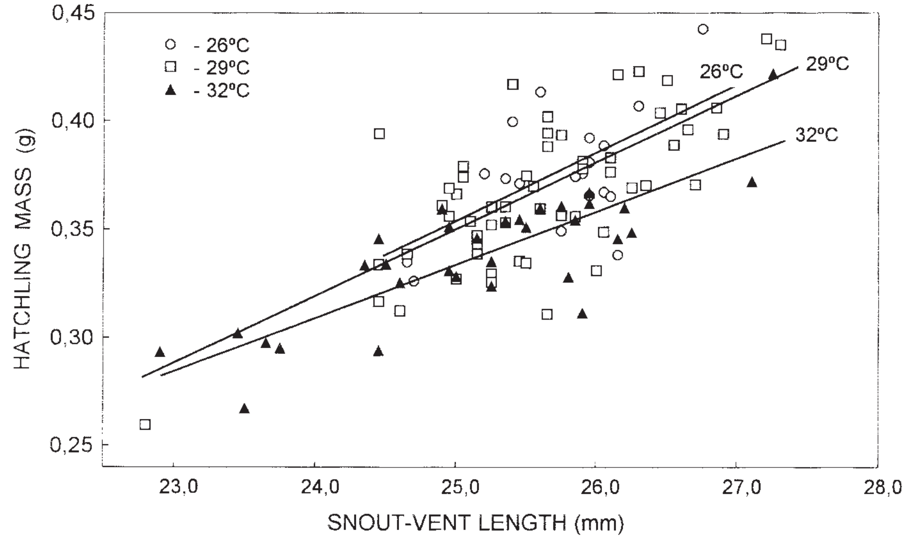

Fig. 1. Relationship between snout-vent length and mass of hatchling *Podarcis muralis* from eggs incubated at 26, 29, and 32°C.

TABLE 2. Loading of the first two axes of a principal component analysis on six morphometrical variables[1]

Variable	Factor loading	
	PC1	PC2
Hatchling mass	0.582	0.379
Tail length	**0.776**	0.001
Head length	**0.827**	0.157
Head width	0.611	0.180
Forelimb length	0.051	**0.881**
Hindlimb length	0.262	**0.808**
Variance explained	34.49%	27.16%

[1] Size effects were removed in all cases by using residuals from the regressions on snout–vent length. Variables with the main contribution to each factor are in boldface.

ling mass, with log SVL as the covariate; $F_{2,61}$ = 13.748, $P < 0.0001$; 26 > (29, 32), Scheffé test], and on tail length [$F_{2,61}$ = 4.803, P = 0.011; (26, 29) > 32, Scheffé test].

Bilateral asymmetry

Heterogeneity of variances among incubation temperature treatments existed (Levene's tests) for the scaled difference between counts or measurements of both sides of one meristic trait (upper labials, $F_{2,107}$ = 3.367, P = 0.038) and two morphometric traits [eye diameter ($F_{2,107}$ = 6.019, P = 0.003), and hind limb length ($F_{2,107}$ = 5.432, P = 0.006)]. The magnitude of the variances, and therefore the level of asymmetry, of foreleg length increased with increasing incubation temperature, whereas the variance of the number of upper labials followed the opposite tendence, and that of the eye length did not reveal any definite trend. Repeated measures ANOVAs, with side (right–left) as repeated within subject factor and temperature as between subject factor, revealed significant side effect, indicative of directional asymmetry, for the eye diameter ($F_{2,107}$ = 13.397, $P < 0.001$), tympanum diameter ($F_{2,107}$ = 11.004, P = 0.001), and supraciliary scales ($F_{2,107}$ = 5.349, P = 0.023). However, significant side × temperature interaction, indicative of temperature effect on the level of directional asymmetry, was only found for tympanum length ($F_{2,107}$ = 5.757, P = 0.0042), and even in this case hatchlings from the higher incubation temperature were the most symmetrical ones.

Correlates of locomotor performance

Sprint speed of each lizard was repeatable between trial temperatures across incubation temperatures (r = 0.423, $P < 0.001$). Within each trial temperature, speed was positively related to the

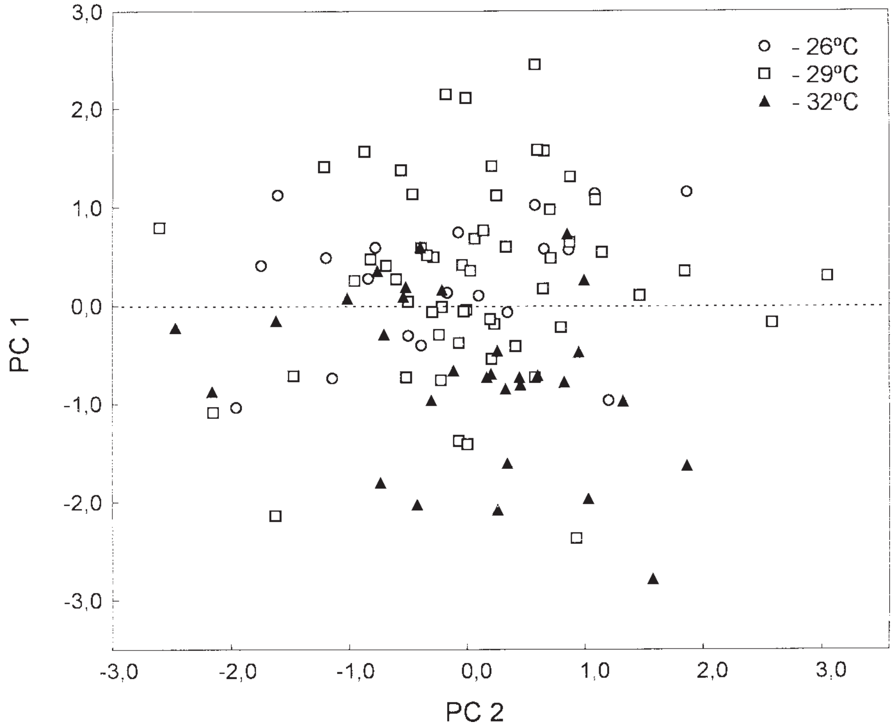

Fig. 2. Position of hatchlings from three incubation temperatures (symbols on the top right corner) in the space defined by the first two axes of a principal component analysis based on six size-adjusted morphological variables.

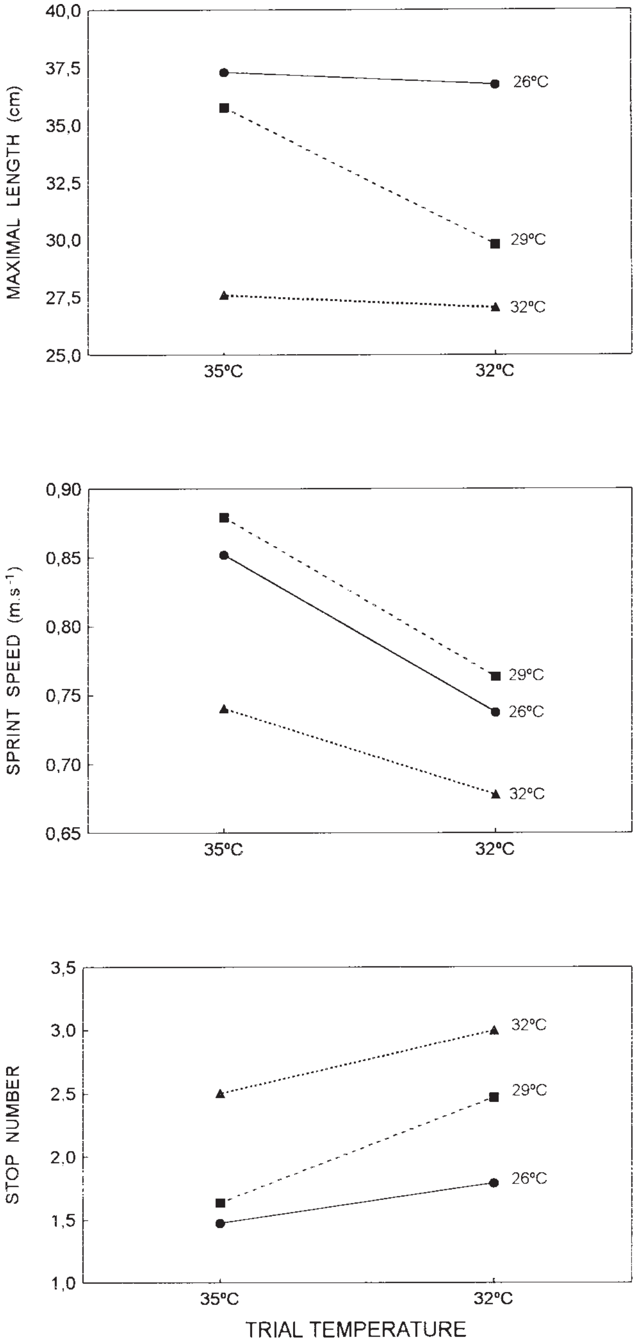

Fig. 3. Effect of thermal environments during the incubation (26, 29, and 32°C) on three aspects of locomotor performance of hatchling wall lizards tested at body temperatures of 32 and 35°C. Mean values of each treatment and trial temperature are represented for maximal length, sprint speed, and stop number.

maximal distance travelled without pause (35°C, $r = 0.534$, $P < 0.001$; 32°C, $r = 0.315$, $P < 0.01$) and negatively to the number of stops in the locomotory sequence (35°C, $r = –0.494$, $P < 0.001$; 32°C, $r = –0.360$, $P < 0.01$). Among the potential covariates essayed, hatchling size (SVL) and tail length were no significantly correlated with none of the running performance traits, either at 32 or at 35°C, probably because of the reduced range of hatchling sizes. Hatchling condition (residuals from the regression of log hatchling mass on log SVL) was uncorrelated with sprint speed, but at the two trial temperatures was positively correlated with the maximal distance covered without stopping ($r = 0.256$ at 35°C, and $r = 0.312$ at 32°C, $P < 0.05$ in both cases), and negatively with the number of complete stopping in the run at 35°C ($r = –0.251$, $P < 0.05$) and 32°C ($r = –0.358$, $P < 0.01$) (Fig. 3).

Among treatment differences in locomotor performance

A repeated-measures ANOVA revealed that sprint speed was affected by incubation treatment and by trial temperature, but no significant interaction between factors was found (Table 3). The same results were obtained by considering a repeated-measures ANCOVA with egg mass, the trait more strongly correlated with running speed, as covariate ($F_{2,65} = 7.07$, $P < 0.01$, for the effect of incubation temperature; $F_{1,65} = 22.16$, $P << 0.001$, for the effect of test temperature). Lizards ran faster at 35 than at 32°C (Fig. 2), and Scheffé tests a posteriori indicated that lizards incubated at 32°C were slower than those incubated at 29 and 26°C. Strong effects of incubation temperature were also evident on the maximal length of running units within trials (shorter for incubations at 32°C), without significant influence of trial temperature, and on the number of stops (more frequent at 32°C), over which test temperature had an important effect (more continuous running at 35°C). Contrary to the sprint speed, considering the effect of the more influential covariate on stop number and maximal length travelled (hatchling condition in both cases) modified results in an important way to denote possible mechanistic links. For both response variables, analyses of covariance showed no significant treatment effect (incubation temperature) when controlled for variation in hatchling condition ($F_{2,65} = 1.847$, $P = 0.166$, for length; $F_{2,65} = 1.580$, $P = 0.214$, for number of stops).

No significant interactions were found between incubation temperature and temperature under which trial were developed, in the ANOVAs (Table 2) or ANCOVAs considering the above mentioned covariates ($P > 0.2$ in all cases) for any of the locomotor traits considered.

TABLE 3. Effects of incubation temperature on locomotor performance of hatchling Podarcis muralis *tested at 32 and 35°C*[1]

Incubation temperature	Locomotor performance					
	Speed ($m \cdot s^{-1}$)		Length (cm)		No. of stops	
	32°C	35°C	32°C	35°C	32°C	35°C
26°C (N = 19)	0.74 ± 0.11	0.85 ± 0.18	36.74 ± 11.84	37.29 ± 10.12	1.79 ± 1.08	1.47 ± 1.02
29°C (N = 30)	0.76 ± 0.14	0.88 ± 0.16	29.78 ± 9.68	35.77 ± 12.08	2.47 ± 1.19	1.63 ± 1.40
32°C (N = 20)	0.68 ± 0.17	0.74 ± 0.12	27.05 ± 12.73	27.60 ± 11.43	3.00 ± 1.86	2.50 ± 1.39
Between subjects	$F_{2,66}$ = 5.137** (29)(32)		$F_{2,66}$ = 4.898** (29)(32)		$F_{2,66}$ = 4.679* (29)(32)	
Within subject	$F_{1,66}$ = 22.502*** 35>32		$F_{1,66}$ = 2.747 ns		$F_{1.66}$ = 10.373** 32>35	
Interaction	$F_{2,66}$ = 0.698 ns		$F_{2,66}$ = 1.861 ns		$F_{2,66}$ = 0.872 ns	

[1] Performance variables (presented as mean ± 1 sd) are sprint speed, maximal distance between stopping, and total number of stops in a single trial. The F ratios correspond to repeated-measures ANOVAs with incubation temperature as between-subject factor and the corresponding performance trait as within-subject factor. Parentheses identify significant differences between treatments, as determined by Scheffé's tests a posteriori.
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Early growth

Across incubation temperatures, hatchling size was influenced by initial egg mass ($r = 0.521$, for SVL; $r = 0.697$, for hatchling mass; $P < 0.001$ in both cases), and these correlations remained, even if attenuated, at the age of 20 days ($r = 0.367$, $P < 0.01$; $r = 0.445$, $P < 0.001$, respectively). Hatchling condition (residuals from the regression of log mass on log SVL) was also positively influenced by initial egg mass ($r = 0.446$, $P < 0.001$). Growth rate (in mass) in the initial 20-day period (log mass at day 20 minus log hatchling mass) was positively related to hatchling size (SVL; $r = 0.272$, $P < 0.05$) (Fig. 4).

Repeated-measures ANCOVAs (with log egg mass as covariate), were used to analyse the effect of sex and incubation temperature (fixed between subject factors) on SVL and mass (both log transformed) at

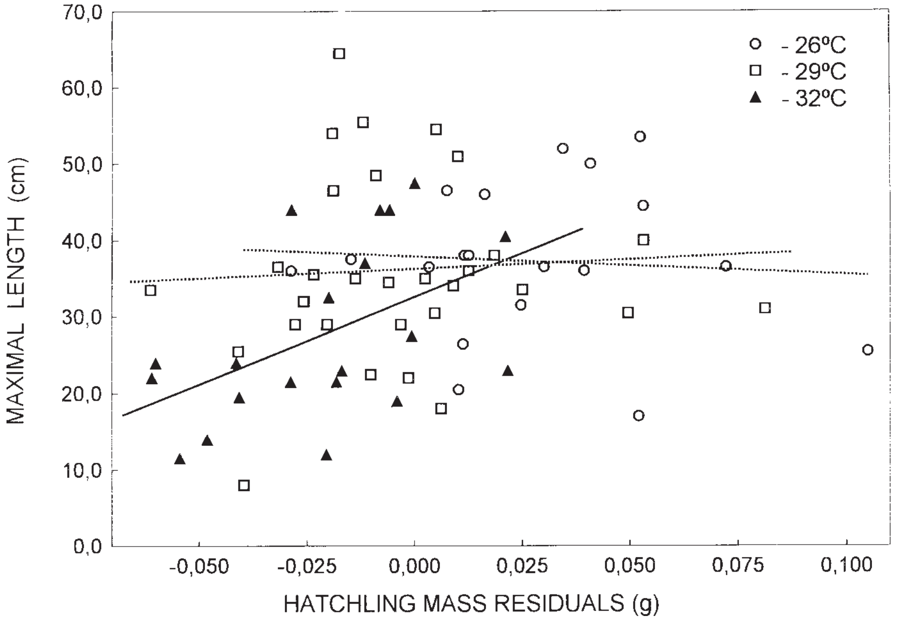

Fig. 4. Maximal length covered without stopping by hatchling wall lizards from eggs incubated at 26, 29, and 32°C as a function of their mass condition (residuals from the regression on snout-vent length). Lizards were tested at body temperature of 35°C. Least-squares regression lines were represented for all the incubation treatments, but the slope was only different from 0 for hatchlings incubated at 32°C (however, an analysis of covariance did not reveal significant differences among the slopes).

TABLE 4. Effects of sex and incubation temperature on hatchling growth during the first 20 days[1]

		Incubation temperature					
		26°C		29°C		32°C	
Sex	Trait	0-day	20-day	0-day	20-day	0-day	20-day
Male	SVL (mm)	25.10 ± 1.45	28.38 ± 1.58	25.13 ± 0.69	29.08 ± 1.24	24.72 ± 0.80	27.86 ± 1.54
	Mass (g)	0.383 ± 0.055	0.525 ± 0.067	0.354 ± 0.032	0.572 ± 0.109	0.330 ± 0.014	0.504 ± 0.092
Female	SVL (mm)	26.48 ± 0.63	29.73 ± 0.88	26.18 ± 0.88	29.73 ± 1.26	25.89 ± 1.13	29.11 ± 2.11
	Mass (g)	0.412 ± 0.047	0.580 ± 0.063	0.377 ± 0.038	0.586 ± 0.116	0.349 ± 0.032	0.558 ± 0.163
Effects	SVL	Sex	$F_{1,60}$ = 15.946***				
		Temperature	$F_{2,60}$ = 1.933 ns				
		Within subject	$F_{1.60}$ = 878.973***				
	Mass	Sex	$F_{1,60}$ = 2.909 ns				
		Temperature	$F_{2,60}$ = 3.375* (26,29)32				
		Within subject	$F_{1,60}$ = 356.297***				

[1] Data on snout–vent length (SVL) and mass are presented as mean ± sd and analysed with repeated measures ANCOVAs (with egg mass as covariate for both hatchling SVL and mass; all variables log transformed) with sex and incubation temperature as between subject factors and size at days 0 and 20 as within subject factor.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

0 and 20 days (Table 4). Growth was evident both in SVL and mass (highly significant effect for the within subject factor), but the effect of sex was significant only in the SVL (females larger than males), and that of incubation temperature was significant in mass (hatchlings incubated at 32°C were lighter than those incubated at 26 and 29°C).

DISCUSSION

The present data provide evidence for a pervasive effect of incubation temperature on hatchling phenotype in the lizard *P. muralis*, reinforcing the results of previous studies on the same population (Van Damme et al., '92; Ji and Braña, 2000a), as well as on some other reptiles (reviews in Deeming and Ferguson, '91; Shine and Harlow, '93; Overall, '94). The persistence of phenotypic effects of the incubation environment through the organism's life and their influence on relevant life history traits (age and size at maturity and age-specific survival schedules, for example) are perhaps the main issues to be addressed in order to understand the ultimate influence of variation in thermal plastic traits (Gutzke and Packard, '87; Shine et al., '97b). The nature of the traits exhibiting among treatment differences (size, morphometry, performances in locomotion and growth) in our study strongly suggests that incubation temperature could have long-term effects and fitness consequences. On the other hand, hatchlings were evaluated for morphological characteristics and tested for locomotor performance soon after hatching so that effects can be certainly attributed to incubation treatment. With respect to burst speed, we have found lowest performance for hatchlings incubated at 32°C, and better overall speed across incubation temperatures was obtained in trials conducted at 35°C than at 32°C. The lack of significant interactions between trial temperature and incubation temperature for any of the performance traits revealed the lack of acclimation effects and suggest that results could be repeatable for locomotor performance at other suitable temperatures. In fact, results of our analyses of locomotor capacity and early growth of the hatchlings are absolutely coherent with those of Van Damme et al. ('92) for two-month old lizards of the same population. All together, these analyses demonstrated that size differences established as a consequence of variation in the incubation temperature remained 20 days after hatching and were also apparent in two-month old lizards. Moreover, lizards incubated at high temperatures persisted to be slower (size-corrected sprint speed) over the two-month period than those incubated at low temperatures (Van Damme et al., '92). Similarly, Shine ('95) reported that the phenotypic effects of incubation temperature persisted for at least two months in the scincid *Bassiana duperreyi*, and Alberts et al. ('97) documented the persistence during the first year of differential growth rates in hatchlings of the iguanid *Cyclura nubila* incubated at different temperatures. Both initial size and early growth rates would imply either anticipating the age at first reproduction (see Galán, '96, for a neighbour population of *P. bocagei*) or raising the size at maturity, and are probably direct fitness correlates.

Incubation temperature does not affect hatchling phenotype in a continuous way, but for most of the analysed traits a critical threshold seems to exist between 29 and 32°C, so that hatchlings incubated at 32°C exhibited differences in morphometry or performance versus those incubated at lower temperatures. The existence and approximate position of the threshold for the main detrimental effects is highly consistent with previous studies on the same population (Van Damme et al., 1992; Ji and Braña, 2000a). It is worth noting, in particular, the consistency of the results for morphometric and performance traits, and this allows examining the possibility that performance (e.g., locomotor capacity) could be indirectly influenced by incubation temperature through its effect on morphology. Hatchlings incubated at 32°C were smaller than those developed at higher temperatures and had lower mass residuals and reduced limbs and tail, and could therefore be expected to have reduced speed (e.g., Garland, '85; Miles, '94; Bauwens et al., '95; Miles et al., '95). Poor locomotory performance of hatchlings from the 32°C incubation treatment could partly reflect morphological limitations, but also incorporates behavioural and energetical components. For example, the high frequency of stops in each trial is a behavioural trait that clearly affected burst speed (in fact they are negatively correlated), and the poor condition status of hatchlings incubated at 32°C is an important correlate (perhaps causal) of their syncopated running pattern. This argues against the uniqueness of morphological differences as the causative factor for the poor performances associated with the highest incubation temperature and suggests more general effect reflecting overall unbalanced phenotypes.

Recent studies on between sex differences in the thermal optima for embryonic development (Gutzke and Crews, '88; Shine et al., '95, '97b), provide an additional interest in testing these differences in order to understand the evolution of temperature-dependent sex determination (Charnov and Bull, '77; Janzen and Paukstis, '91). All conditions of incubation used in our experiments yielded an equal sex ratio (Ji and Braña, 2000a), and the evidence that sexes could differ in their phenotypic responses to incubation temperature (i.e., significant interactions between sex and incubation temperature for phenotype components, or complex response variables) was very weak and limited to a few morphometric traits (head size and femur length).

Bilateral symmetry is considered to reflect developmental stability and to have a positive relationship with several fitness components (Møller, '97; Møller and Swaddle, '97; but see Clarke, '98). Fluctuating asymmetry is thought to be associated with environmental stress during development (Palmer and Strobeck, '86; Leary and Allendorf, '89), so that high levels should reflect unsuitable developmental conditions, and identify comparatively unbalanced phenotypes. In addition to this indicative value, it seems reasonable to think that high bilateral asymmetry could cause troubles in many functions involving bilateral organs, such as locomotion or several kinds of sensorial perception, and could therefore be detrimental by itself, and not only as an overall indicator of poor quality phenotypes. Therefore, differences in fluctuating asymmetry among hatchlings from different incubation temperatures could be indicative of comparative quality. Our results did not show an absolutely coherent pattern for all the studied traits (what is a very frequent pattern in studies of fluctuating asymmetry; see, e.g., Palmer and Strobeck, '86; Clarke, '98), but clearly indicated a high level of asymmetry (both fluctuating and directional) in the whole sample. Differences among incubation temperatures in the level of asymmetry at the hatching stage were generally weak and sometimes inconsistent with the prediction that environmental stress associated with higher incubation temperatures might produce the highest level of asymmetry. The discrepancy could be partly explained by the higher mortality experienced by embryos developing at 32°C (Ji and Braña, 2000a), likely removing the most unbalanced phenotypes. One additional consideration is that, because all of the experimental temperatures are quite high, perhaps near (for 26 and 29°C) or above (32°C) the limit experienced in natural incubations in the field, this could actually represent stressing developmental conditions for all treatments. In fact, thermal stresses producing abnormal development can sometimes be within the temperature range experienced by animals in nature (Cossins and Bowler, '87).

Our results agree with previous studies in recognizing the incubation thermal environment as a source of phenotypic variation in lizards, potentially important in natural conditions, but the implications of this variation for offspring fitness in field conditions remain largely unexplored, and offer considerable potential for future work. For example, we know that *P. muralis* reproduce over almost four months in spring-summer (April to July) and each female lays 1–3 clutches in this period of progressively warmer thermal environment. Larger (older) females start reproduction earlier and produce both more clutches and larger eggs (Ji and Braña, 2000a,b); moreover, all females tend to produce larger eggs in their first clutch. In this situation, date of egg laying and behavioural maternal effects (by nest site selection; Resetarits, '96; Roosenburg, '96) could interact with egg size (the main maternal effect; Bernardo, '96a,b) and genotype in determining hatchling size. Therefore, a considerable fraction of the variation in hatchling size, which is likely a major predictor of fitness, seems to be determined by the incubation environment and maternal effects, and therefore natural selection operates on a trait whose evolutionary response could be altered or delayed (Kirkpatrick and Lande, '89; Lande and Kirkpatrick, '90; Cowley and Atchley, '92). This could set a critical stage of selection acting on hatchling size (or more generally, on hatchling phenotype) in female's selection of nest sites to attain optimal environments for embryogenesis (Packard, '91; Shine and Harlow, '93; Shine et al., '97b). Also, prolonged egg retention and changing thermal preferenda of gravid females (as already demonstrated for *P. muralis*; Braña, '93) could have evolved because of the developmental advantages associated with the high thermal homogeneity and optimality that gravid females provide to embryos, and this has been proposed as a possible ultimate cause for the evolution of reptilian viviparity (Shine, '95).

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