Intraspecific variation of preferred temperatures in the NE form of *Podarcis hispanica**

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Podarcis hispanica* *is, in fact, a complex of several forms and ecophysiological traits previously investigated in one of them may be no longer generalisable to the others. Intraspecific variation of preferred temperatures (Tp) was analysed in a population of the NE form of* P. hispanica* *and compared with data on other forms coming from the literature. Adult lizards were collected in May, measured (SVL), weighed and exposed to a thermal gradient at nine time intervals. Tp changed with sex (males > females), size (positive correlation with body mass) and, more weakly, with time of day (early morning > mid morning and mid-day > afternoon). Two females decreased their Tp after egg-laying. Results corroborate previous studies demonstrating diel adaptability of Tp in lacertids and strong dependence on the individual's condition. Tp should be interpreted as a compromise between different pressures including gonadal changes, embryo development and social behaviour. Furthermore, in a comparison restricted to males, the NE form attained lower Tp than other Iberian* Podarcis *not directly related in the phylogeny, which may indicate changes at evolutionary level.*

Keywords: thermal ecology, preferred temperatures, sex variation, diel variation, pregnancy, *Podarcis hispanica**, Iberian Peninsula.

Introduction

Once considered a single species, *Podacis hispanica** from the Iberian Peninsula, SE France and N Africa constitutes, in fact, a species complex, paraphyletic with *P. bocagei*

and *P. carbonelli* (Harris & Sá-Sousa 2001, 2002; Harris *et al.* 2002; Pinho *et al.* 2003, 2006). Up to 9-10 forms of this complex, 6-7 of them in the Iberian Peninsula, have been characterised based on nuclear and mitochondrial markers (see previous references) and, some of them, are also supported by morphological (Geniez 2001, Sá-Sousa *et al.* 2002) and behavioural (Barbosa *et al.* 2005) evidence. Since considerable evolutionary divergence between them has been found (i.e. 10-12% in cyt-*b* mtDNA equivalent to 6-7 My, Harris *et al.* 2002), those ecophysiological traits previously investigated in one of these forms may be no longer generalisable to the others.

Preferred body temperature (Tp), in the absence of thermoregulatory constraints, correlates with several physiological optima in lizards (Huey & Bennet 1987, Bauwens *et al.* 1995). Two kinds of Tp variability should be considered (Castilla *et al.* 1999): within a population (short time scale) and among populations/species (evolutionary scale). On the other hand, individual lacertids are able to change their Tp in response to temporal variation within their life such as seasonal changes, reproductive condition and feeding status (Castilla *et al.* 1999, Carretero *et al.* 2005). In contrast, this trait appears to be evolutionarily conservative in lacertids responding slowly to directional selection, hence, Tp remaining similar between different populations of the same species (Patterson & Davies 1978; Van Damme *et al.* 1989, 1990; Gvozdík & Castilla 2001; Carretero *et al.* 2005; but see Scheers & Van Damme 2002) and only varying between species (Bauwens *et al.* 1995). Such variation could take place between the different Iberian forms of *Podarcis* since considerably variation is found in thermal environment of both their present ranges and their putative areas of origin (Sá-Sousa 2000, 2001; Harris *et al.* 2002).

Thus, in this paper we aim 1) to analyse the patterns of the intraspecific variation of Tp within a population of the NE form of *P. hispanica** and 2) to make a preliminary assessment of the conservativeness of Tp in the *P. hispanica** species complex by comparing the values obtained with those available for other forms in the literature.

Materials and Methods

Sixteen adult *P. hispanica** (9 males and 7 females) were collected in a thermo-mediterranean site from NE Iberia (Bellaterra, Cerdanyola del Vallés, UTM 31T DF2395, 150 m altitude) dominated by garrigue patching with *Pinus halepensis* and *Quercus ilex* forests in May 2000, coinciding with the peak of oviposition in this area (Llorente 1988, Kaliontzopoulou *et al.* 2004). Lizards were kept in individual 0.5 × 0.4 × 0.3 m terraria during not more than 2 weeks with food and water provided *ad libitum* and then released in the site of capture after the experiments. Immediately before each test, the lizard was measured (snout-vent length, SVL) to the nearest 0.05

mm with a digital calliper and weighed to the nearest 0.01 g with a digital balance. Each lizard was individually exposed to a photothermal gradient (~ 20-45 ºC, 0.5 × 0.5 × 1.5 m length) produced by a 100-w reflector bulb fixed 15 cm above the *substratum*. The room of the experiments was fully exposed to sun light, hence, natural photoperiod was maintained. Tp was measured by inserting a k-termocouple probe associated with a digital thermometer (Digitron® 3208K, accuracy 0.01 ºC) in the cloaca. Body temperatures were recorded only for active lizards during a single day at nine consecutive intervals (Fig. 1, Table 1) distributed throughout the period of diel activity observed in the field (Llorente 1988). Time interval between two consecutive measurements was 1 hour. A limited subset of females (n = 2) was analysed before and after egg-laying in terraria.

Data were not transformed since distributions did not deviate from normality (Kolmogorov-Smirnov tests, *P* > 0.05 in all cases), were homocedastic (univariate Levene tests and multivariate Box M, *P* > 0.05 in all cases) and variances and means were uncorrelated. Since measures were repeated for the same individual (for each interval and, in two females, before and after egg-laying) statistical analysis was based on Analysis of Variance for Repeated Measures (ANOVAR) of Tp with class (males, pregnant females, non-pregnant females) as between subject factor and time interval as within subject factor. Sphericity assumption of ANOVAR was tested by means of Mauchley's tests.
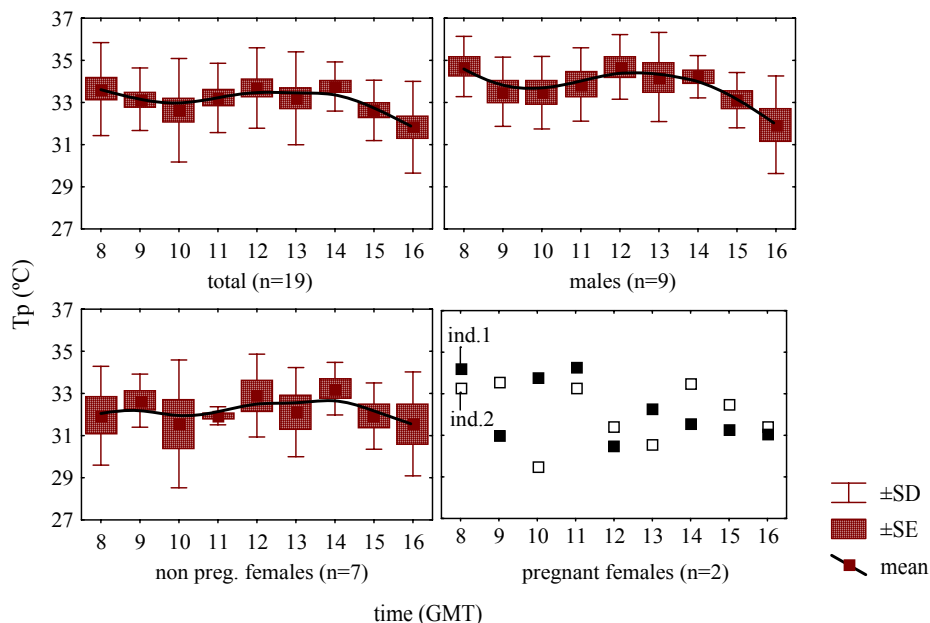

Fig. 1. Temporal variation of Tp considering the three classes and pooled.

Table 1. Descriptive statistics of Tp considering lizard classes and time intervals.
* means of 9 time intervals.

class	time	n	mean ± SE	median	min.	max.
males	8	9	34.71 ± 0.48	35.30	32.5	36.7
	9	9	33.51 ± 0.55	33.50	30.7	36.7
	10	9	33.47 ± 0.57	32.60	32.0	37.3
	11	9	33.86 ± 0.58	34.10	30.6	36.7
	12	9	34.69 ± 0.51	34.80	32.0	36.5
	13	9	34.21 ± 0.71	35.50	29.9	36.4
	14	9	34.22 ± 0.33	34.50	32.8	35.8
	15	9	33.11 ± 0.44	32.70	31.9	36.0
	16	9	31.94 ± 0.77	31.60	29.0	35.7
	total*	9	33.75 ± 0.24	33.85	29.0	37.3
non preg. females	8	7	31.94 ± 0.89	33.00	28.8	34.5
	9	7	32.66 ± 0.48	32.40	31.1	34.4
	10	7	31.56 ± 1.15	32.00	26.0	36.1
	11	7	31.94 ± 0.16	32.00	31.3	32.7
	12	7	32.90 ± 0.74	32.80	30.0	35.3
	13	7	32.11 ± 0.80	31.80	28.7	34.9
	14	7	33.23 ± 0.47	33.20	31.7	35.5
	15	7	31.93 ± 0.59	31.50	29.3	33.9
	16	7	31.56 ± 0.93	33.10	28.0	34.0
	total*	7	32.20 ± 0.31	32.12	26.0	36.1
pregnant females	8	2	34.75 ± 0.45	34.75	34.3	35.2
	9	2	33.30 ± 1.30	33.30	32.0	34.6
	10	2	32.65 ± 2.15	32.65	30.5	34.8
	11	2	34.80 ± 0.50	34.80	34.3	35.3
	12	2	31.95 ± 0.45	31.95	31.5	32.4
	13	2	32.45 ± 0.85	32.45	31.6	33.3
	14	2	33.55 ± 0.95	33.55	32.6	34.5
	15	2	32.90 ± 0.60	32.90	32.3	33.5
	16	2	32.25 ± 0.15	32.25	32.1	32.4
	total*	2	33.18 ± 0.06	33.18	30.5	35.3

Results

Table 1 shows the descriptive statistics by class and time interval. Overall Tp (means of 9 intervals, pregnant females excluded) was 33.07 ± 0.27 ºC (26.0-37.3). Nevertheless, ANOVAR revealed substantial variation with both class and time in-

Table 2. Results of the ANOVARs. Analysis restricted to females produced identical results with and without covariate.

2-way ANOVAR	F	d.f.	P
sex	15.91	1, 14	0.001
time (R)	2.16	8, 112	0.036
sex × time	0.71	8, 112	0.682
Mauchley's sphericity test	$\chi^2 = 37.10$	35	0.37
2-way ANOVAR (weight cov.)	F	d.f.	P
sex	8.20	1, 13	0.01
time (R)	2.16	8, 112	0.036
sex × time	0.71	8, 112	0.682
Mauchley's sphericity test	$\chi^2 = 37.10$	35	0.37
2-way ANOVAR (females)	F	d.f.	P
pregnancy (R)	3.30	8, 8	0.055
time (R)	0.47	1, 1	0.618
pregnancy × time	0.28	8, 8	0.183

terval (Fig. 1, Table 2). Tp values were high in the early morning, decreased in the mid-morning, rose in the early afternoon and decreased again in the late afternoon. Males selected for higher temperatures than females and, in the two cases analysed, females decreased their Tp after egg-laying, although such variation was only marginally significant (Fig. 2, Table 2).

Furthermore, partial correlation analysis with all individuals except pregnant females detected significant positive correlation of individual mean Tp with weight ($Rp_{13} = 0.63$, *P* = 0.01) but not with SVL ($Rp_{13} = -0.49$, *P* = 0.07, n.s.) (Fig. 2). Similar results were obtained for minimum Tp reached by individual in the nine time intervals (SVL $Rp_{13} = 0.55$, *P* = 0.03; weight $Rp_{13} = -0.25$, *P* = 0.37, n.s.). Thus, we repeated both ANOVARs using body weight as a covariate but the results of comparisons remained the same (Table 2).

Discussion

These results corroborate recent studies on other lacertids demonstrating strong dependence of Tp on the individual's condition as well as weaker but significant diel adaptability (Castilla *et al.* 1999 and references therein, Gvozdík & Castilla 2001, Carretero *et al.* 2005).
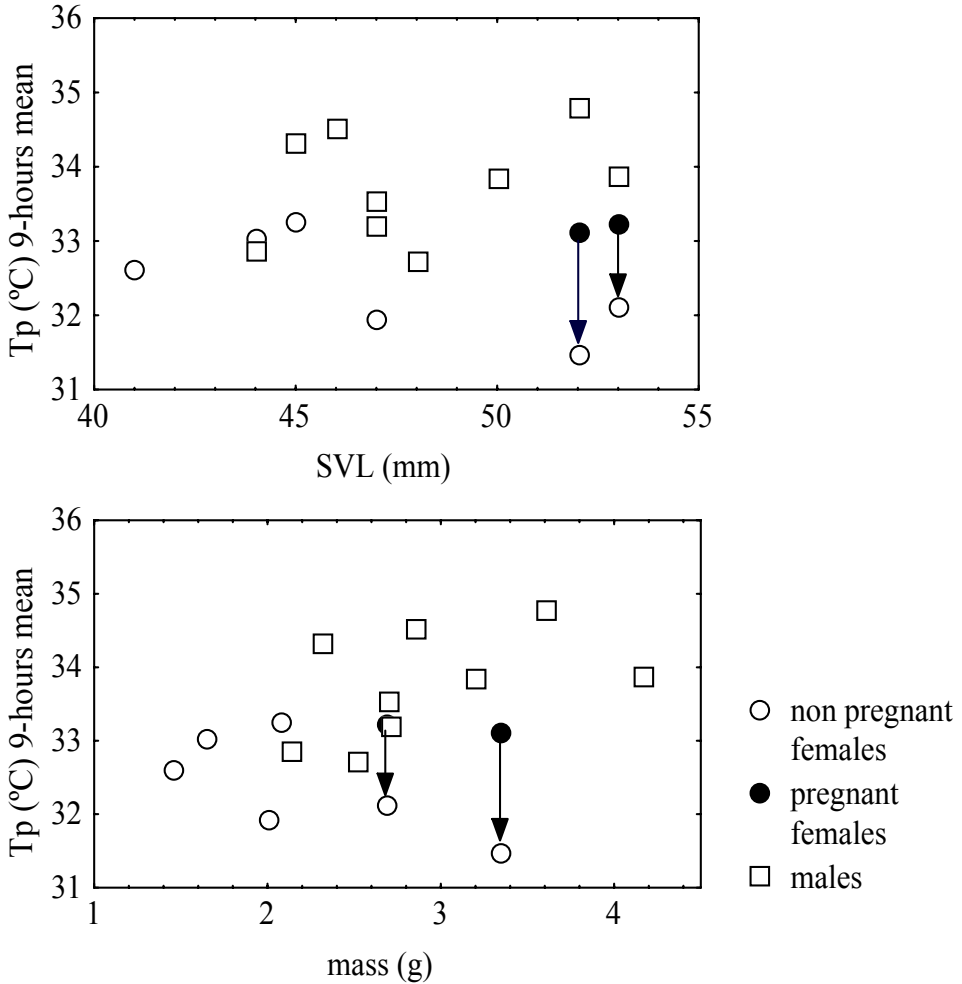
Fig. 2. Relationships between Tp and body size. Arrows indicate changes after egg-laying.

First, lacertids tend to raise Tp in the early morning when environmental temperatures are low and thermoregulation becomes a priority in relation to other requirements (Rismiller & Heldmaier 1982, Tosini & Avery 1994, Carretero *et al.* 2005). The results of this study indicate that the same does not apply to the late afternoon, at least in *P. hispanica**.

Moreover, pregnant female Tp usually reflects the optimum for embryonic development rather than for the physiology of the female itself (Carretero *et al.* 2005). In contrast, with *L. vivipara* (Heulin 1987, Van Damme *et al.* 1987, Gvozdík & Castilla 2001, Carretero *et al.* 2005), *P. hispanica** females decreased

Tp after egg-laying. Although low sample size does not allow a general picture to be drawn, gestation period is much shorter in *Podarcis* spp. than oviparous *L. vivipara* (Braña *et al.* 1991), suggesting lower constraints for embryo development. Nevertheless, other physiological processes may be involved since non-pregnant females still showed lower Tp than males. For instance, it has been suggested that the spermatogenic cycle in males would need higher temperatures (Carretero *et al.* 2005), especially in the beginning of the reproductive season (Gavaud 1991, Carretero *et al.* submitted).

On the other hand, influence of relative body size also occurred within adults suporting previous suggestions that small individuals are thermally more opportunistic (Carretero & Llorente 1995, Carretero *et al.* 2005) and/or compensate for evaporative loss (Bowker 1993, Lorenzon *et al.* 1999). The fact that body mass and not body length itself is the main responsible for such variation does not exclude either of both hypotheses and further research is needed to distinguish between these two factors. Essentially, discrepancies between mass and length in lizards are due to regenerated tails and fat reserves. Both parameters involve changes in body condition which should be the putative proximal factor to be explored.

Obviously, after all this evidence any interpopulation analysis should avoid using pooled data but perform at least class-by-class comparisons (see Carretero *et al.* 2005). Male Tps observed here (mean ± SE: 33.75 ± 0.24, median: 33.85 ºC) were lower than those published for other Iberian members of the complex (*P. carbonelli* median 35.15 ºC; *P. hispanica** NW form: median 34.40 ºC; *P. (h.) atrata* from Columbretes islands: median 34.50 ºC; Castilla & Bauwens 1991, Bauwens *et al.* 1995) and even than the Middle European *P. muralis* (median 34.15 ºC; Bauwens *et al.* 1995). Since lab methods were essentially the same, then assuming the comparativeness of the results, it should be concluded that Tp differences arose from old, independent evolutionary trajectories under different thermal conditions. In fact, available phylogenies indicate that, whereas *P. carbonelli* and the NW form of *P. hispanica** are related groups originated in W Iberian Peninsula (Harris & Sá-Sousa 2001, 2002), the NE form of *P. hispanica** is deeply divergent form all other Iberian *Podarcis* (except those from the SE, Pinho *et al.* 2006) and occupies the northernmost range (including France, Catalonia, Ebro Valley, Basque Country and N Castilian Plateau, Geniez 2001 unpubl. data). Nevertheless, the same evidence indicates a weak separation between *P. (h.) atrata* and the NE form of *P. hispanica* (Harris & Sá-Sousa 2002). Further studies including the other forms and replicating the same methodology are currently being carried out (unpubl. data) to corroborate these preliminary conclusions.

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