

Effects of gravity on the locomotor performance and escape behaviour of two lizard populations: the importance of habitat structure

Pablo Iraeta^{1,3)}, Alfredo Salvador²⁾, Camila Monasterio^{1,2)}
& José A. Díaz¹⁾

(¹ Departamento de Zoología y Antropología Física (Vertebrados), Facultad de Biología, Universidad Complutense, E-28040 Madrid, Spain; ² Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, E-28006 Madrid, Spain)

(Accepted: 24 July 2008)

Summary

In lizards, locomotor costs of gravity may depend on habitat structure and refuge availability. We compared the locomotor performance and escape tactics in the laboratory, before and after oviposition, of two populations of *Psammotromus algirus* separated by 700 m altitude. When gravid, females escaped using a larger number of slower and shorter runs, and had lower temperatures at the time of trial, than after oviposition. Some of these effects differed between populations: when gravid, but not after oviposition, low-elevation females ran shorter distances at a slower average speed than high-elevation ones. Low-elevation females laid their clutches earlier than high-elevation ones, which conditioned their lower speed when gravid in simultaneous running trials. However, their escape distances were still shorter after controlling for the effects of temperature and laying date. In the field, refuge availability was lower at the low-elevation site, where females spent more time inside refuges and perching above ground. The shorter escape distance of low-elevation females may, thus, represent a behavioural response to minimize detectability, especially if predator avoidance depends primarily on whether or not the lizard is seen by the predator. Such behavioural adjustments might inhibit selection for evolutionary shifts in the performance of gravid females.

Keywords: crypsis, predator avoidance, *Psammotromus algirus*, reproductive costs, body temperature.

³⁾ Corresponding author's e-mail address: piraeta@bio.ucm.es

Introduction

Reproducing females carry a burden of eggs or young that is likely to decrease their survival if the impairment of locomotor performance suffered by gravid females makes them more vulnerable to predators (Vitt & Congdon, 1978; Shine, 1980; Downes & Shine, 2001). Such reduction, which can be physical (i.e., due to the burden of the clutch), physiological (i.e., associated with the endocrinological and physiological changes caused by gravity, such as shifts in muscular strength, metabolic capacity or motivation to run), or both (Shine, 1980; Bauwens & Thoen, 1981; Sinervo, 1999; Olsson et al., 2000; Shine, 2003), should, therefore, be regarded as a crucial cost of reproduction (Williams, 1966; Reznick, 1985; Sinervo & DeNardo, 1996). However, decreased performance may or may not impose a significant decrease in fitness (Arnold, 1983) depending on the ecological and behavioural context in which gravid females dedicate their time and energy to reproduction. Thus, any understanding of the functional significance of locomotion will be incomplete without data on how organisms behave in nature (Irschick & Garland, 2001).

Females from lizard populations occupying open or unsafe habitats might select higher temperatures during gravidity (Blouin-Demers & Weatherhead, 2001; Lourdais et al., 2008) in order to minimize locomotor impairment, given the thermal dependence of locomotor performance (Bauwens et al., 1995). However, increased basking can also influence predation risk by increasing an individual's exposure time to predators. On the other hand, females often select lower temperatures during pregnancy due to constraints on thermoregulating opportunities, if the behavioural changes from flight to crypsis associated with pregnancy preclude careful thermoregulation (Braña, 1993), or to the negative effects of high temperature on the phenology or phenotype of offspring (e.g., Mathies & Andrews, 1997; Le Galliard et al., 2003; Ji et al., 2006, 2007). Therefore, the benefits of thermoregulatory shifts from a locomotor perspective would have to outweigh the effects on offspring phenotype and predation risk. Another possibility would be to reduce egg retention to shorten the timespan during which gravid females expose themselves to an increased predation risk in unsafe habitats.

Finally, females could modify their antipredator behaviour during gravidity in response to between-population differences in critical aspects of the environment like vegetation cover or refuge availability (Martín & López,

1995). As behaviour is suggested to exhibit a greater level of plasticity than morphology or physiology, the first level of an animal's response to environmental change is likely to be behavioural, and these behavioural changes can actually buffer natural selection pressures (Huey et al., 2003). However, evolution of local adaptation may result in different behaviours during gravidity for females from different populations. This reinforces the need of combining ecological habitat data with field observations of behaviour in order to interpret the performance measurements obtained in the laboratory (Irschick & Garland, 2001). Thus, gravid females could rely more on crypsis than males or non-gravid females to reduce their conspicuousness (Vitt & Congdon, 1978; Bauwens & Thoen, 1981; Cooper et al., 1990), they could modify their escape strategy (e.g., by varying the degree of wariness, approach distance, number of runs, or mean distance run), or they could change the proportion of maximal sprint speed they use when fleeing (Husack, 2006).

In this study, we evaluate the effects of gravidity on the locomotor performance of two populations of a widespread Mediterranean lacertid lizard, the large psammodromus *Psammodromus algirus*, separated by an altitudinal gradient of 700 m in central Spain. These two populations differ in their phenology (low-elevation females tend to breed earlier than high-elevation ones) and in their reproductive strategy along the egg size vs. number trade-off (Iraeta et al., 2006, 2008): the high-elevation females produce more but smaller eggs than the low-elevation ones. We take advantage of this natural variation to compare the costs of reproduction incurred by gravid females. For that purpose, we compare the locomotor performance and escape tactics in the laboratory of females from both populations before and after oviposition, while considering the effects of morphology, laying date and temperature selection. We also quantify and compare the mean distance to the nearest refuge (i.e., shrub in which a lizard could seek shelter) as a simple index of habitat structure, and we evaluate habitat use and antipredatory behaviour in the field to frame the laboratory measurements in their appropriate ecological context.

Material and methods

Study species and study areas

Psammodromus algirus is a medium-sized (adult snout–vent length 60–90 mm; mass 6–16 g), ground-dwelling, heliothermic lacertid that in-

habits shrub and woodland habitats on both margins of the Western Mediterranean basin (Arnold, 1987; Díaz & Carrascal, 1991). It is a widely distributed species which shows variation in life-history traits across its range (Díaz et al., 2005; Iraeta et al., 2006, 2008; Díaz et al., 2007). Courtship and egg-laying occur between April and June, and offspring hatch in August–September (Veiga & Salvador, 2001).

Our low-elevation study area was located at ‘El Pardo’ (Madrid, central Spain: 40°31’N, 03°47’W; 650 m elevation), a cleared holm oak (*Quercus ilex*) broad-leaved, perennial forest. The high-elevation site was located at Navacerrada (Cerro de la Golondrina, Sierra de Guadarrama, central Spain: 40°44’N, 4°00’W; 1300 m elevation), at a deciduous Pyrenean oak (*Quercus pyrenaica*) forest 32 km by air from the lowland site. For a detailed description of the study areas see Iraeta et al. (2006).

Husbandry of adults

During April and May 2005, we captured adult lizards (either by hand or with a noose) and we transported them on the same day of capture to the laboratory at the Universidad Complutense de Madrid. Lizards were housed in terraria (40×60 cm, 30 cm high) filled with moistened earth averaging 10-cm depth, covered by a leaf litter layer. 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 et al., 2006). Lizards were fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*), dusted with a commercial vitamin and calcium supplement delivered according to manufacturer’s recommendations. Water was available ad libitum. The reproductive status of females was monitored daily to determine the laying date. Overall, 19 females from El Pardo and 13 from Navacerrada, which laid clutches composed entirely of viable eggs (i.e., eggs that succeeded to hatch), were used for the gravid and post-laying running trials. Upon finding the clutch, we counted and weighed all eggs. Details of the husbandry of adult lizards, eggs and juveniles can be found in Iraeta et al. (2006). After all laboratory work was completed, all individuals were released at their sites of capture.

Morphological variables

Since body size (SVL) and hind limb length can determine locomotor performance (Losos, 1990; Bauwens et al., 1995), we measured these morpholo-

gical traits on captured females. Hind limb length (from the tip of the fourth toe, excluding the claw, to the point where the limb connects to the body) was measured to the nearest 0.5 mm by extending the limb over a ruler. Both measurements had high repeatabilities, as shown by a preliminary analysis of thirteen individuals that were measured twice (intraclass correlation coefficients of 0.876 and 0.888 for SVL and hind limb length, respectively; $F_{12,13} > 15$ and $p < 0.001$ in both cases).

Locomotor performance and escape strategy in the laboratory

Running performance was estimated and analyzed following Pérez-Tris et al. (2004). We used a racetrack ($240 \times 30 \times 40$ cm) with a cork floor (to ensure traction) that was marked with perpendicular white bands every 30 cm, dividing the racetrack into six stretches (the 30-cm ends of the racetrack were not considered in the analyses). Each animal was placed on one end of the track and forced to run by tapping near the tail, until it covered three times the racetrack or it refused moving despite tapping. We excluded from the analyses trials in which lizards walked, jumped or showed other unusual behaviours instead of running. Runs were recorded at 30 frames/s using a digital camera (Sony Cyber-shot DSC-V3) placed on a tripod above one end so that the entire racetrack could be viewed on a single image. Video files were transferred to a computer in which speed was measured (in cm/s) by counting the number of frames within each known-distance run and converting them into time.

Each lizard was chased down between one and four times on non-consecutive days both before and after oviposition. This provided a total average ± 1 SE of 2.7 ± 0.2 and 2.9 ± 0.1 measurements per individual before and after laying, respectively, which should provide reliable estimates of locomotor performance. Repeatabilities were high for all measurements except for maximum speed, which showed more variation between trials than the remaining variables (Table 1). Given the behavioural nature of the variables and the temporal separation between measurements, these repeatabilities suggest our results reflect biologically relevant variation in performance.

Because running performance may be influenced by temperature (Bauwens et al., 1995), we registered the cloacal temperature of every lizard with a Miller & Weber quick-reading thermometer immediately before placing it on the racetrack. All temperatures fell within the thermal-performance range

Table 1. Repeatabilities of performance variables with an average sample size of 2.7 (gravid) and 2.9 (post-laying) measurements per individual.

	Average speed		Maximum speed		Number of runs		Maximum distance	
	r_i	p	r_i	p	r_i	p	r_i	p
High-elevation gravid	0.316	0.016	0.092	0.241	0.490	<0.001	0.542	<0.001
Low-elevation gravid	0.535	0.002	0.267	0.083	0.527	0.003	0.568	0.001
High-elevation post-laying	0.456	0.004	0.217	0.095	0.509	0.001	0.352	0.019
Low-elevation post-laying	0.330	0.012	0.207	0.076	0.390	0.004	0.359	0.007

Results of the associated ANOVAs are also shown.

in which the speed is at least 80% of its maximum (28.3–39.1°C; Bauwens et al., 1995), indicating that lizards were not thermally impaired for running.

The evaluation of behavioural escape strategy was achieved using two variables, number of runs (the total number of different sprints, separated by pauses, done by each lizard during a trial) and maximum fleeing distance (maximum distance covered by a lizard during a single run; this must be lower than 180 cm because the dimensions of the racetrack, but this value is high enough for a medium-sized lizard; Martín & López, 2000; Pérez-Tris et al., 2004). To determine running performance, we used average sprint speed (mean speed value for all 30 cm stretches in a running session) and maximum sprint speed (value of the fastest 30 cm stretch in a running session). Principal component analyses with these four variables (results not shown) yielded two factors both for gravid (88.5% of total variance explained) and post-laying females (92.6% of total variance explained): one component was a measure of escape capacity, giving higher scores to females with higher average and maximum sprint speeds, whereas the other component was related to the organization of escape behaviour, giving low scores to few long flights, and high scores to many short runs, independently of the speed (see Pérez-Tris et al., 2004 for a similar result). Therefore, we can conclude that number of runs and maximum fleeing distance are variables which estimate behavioural decisions. However, we subsequently analysed the four original variables rather than the principal component scores because they are easier to interpret and discuss. We used the average of all trials as an estimate of the escape strategy and running performance of each individual before and after oviposition.

Previous studies have pointed out that laboratory measurements of performance may not reflect the real performance of individuals in the field, and that greater integration of laboratory studies of performance and behavioural/ecological studies is needed (Irschick & Garland, 2001; Irschick, 2003). However, laboratory racetracks provide good estimates of the underlying maximum speeds of most lacertids (Irschick et al., 2005). Moreover, we excluded trials in which individuals performed clearly sub-maximally, and we replicated measurements to ensure that estimates of repeatabilities could be conducted (Losos et al., 2002).

Field procedures

To compare space use and antipredatory behaviour under natural conditions, the two study areas were visited regularly by one of us (P.I.) during the 2007 reproductive season (7 May–27 June; 6 days at the high-elevation site and 9 days at the low-elevation site) during the late morning hours (11:00 to 14:00). The researcher walked slowly until a gravid female (which could be clearly identified as such by the extent of abdominal expansion) was sighted. He then noted the height above ground and situation inside or outside refuge (these lizards always seek shelter within dense vegetation patches; Díaz, 1992) of the lizard location at first sighting. During each visit, a different part of the study area was sampled to avoid pseudo-replication. In addition, refuge availability was estimated by measuring the distance to the nearest refuge (i.e., shrub or dense vegetation patch in which a hiding lizard would be difficult to detect and capture) at 50 points separated by 10 m intervals along a 500 m transect in each of the two study areas.

Statistical analyses

To test for treatment effects (site and gravidity) we used a within-subjects MANOVA in which the grouping factor was site of origin and the dependent variables were the differences between pre- and post-laying means for average and maximum running speed, number of runs, and maximum distance ran. Thus, the null hypothesis for the repeated measures effect (gravidity) is that the intercept of the linear model is equal to zero, whereas the factor effect allows testing for the significance of the site \times gravidity interaction (extent to which differences between pre- and post-laying traits are larger

in one population than in the other). To control for the potential confounding effects of body temperature or extent of gravidity, we used MANCOVAs with the difference in mean T_b before and after oviposition or the mean time to lay as the covariates. Finally, and to avoid the loss of power in saturated models due to the large amount of multicollinearity in our data set, we also performed univariate stepwise multiple regressions with the difference between pre- and post-laying means in performance (average and maximum sprint speed) or behaviour (number of runs and maximum distance run) as the dependent variables, and with site, the difference in mean T_b before and after oviposition, and the mean time to lay as predictors. When necessary, data were log-transformed to meet the requirements of parametric tests. All data are given as mean \pm 1 SE.

Results

Locomotor performance and escape strategy in the laboratory

No differences between populations were found in any of the morphological variables studied (Table 2). However, there were differences in clutch size and mean egg mass: females from the low-elevation site produced smaller clutches of larger eggs (Table 2), confirming the pattern found in previous studies (Iraeta et al., 2006, 2008). None of the variables describing escape performance or escape strategy were correlated with any of the morphological or reproductive variables, neither in gravid nor in post-laying females (all p values $>$ 0.2).

Table 2. Mean values (\pm SE) and ANOVAs for morphological data of gravid females from both populations.

	Low-elevation site ($N = 19$)	High-elevation site ($N = 13$)	F	p
SVL (mm)	80.5 \pm 0.8	82.3 \pm 0.9	2.52	0.123
Hind limb length (mm)	39.5 \pm 0.3	39.4 \pm 0.4	0.04	0.843
Clutch size	5.9 \pm 0.2	7.2 \pm 0.4	8.07	0.008
Egg mass (g)	0.46 \pm 0.01	0.42 \pm 0.01	6.45	0.017
Clutch mass	2.71 \pm 0.12	2.94 \pm 0.14	1.55	0.223

For egg mass and clutch mass, $N = 18$ for the low-elevation site because one clutch was slightly dehydrated and had lost weight when the eggs were encountered.

Overall, females modified their locomotor performance and escape strategy when gravid (Table 3, Model 1 MANOVA: $p < 0.001$). Moreover, the effects of gravidity differed significantly between populations (gravidity \times site interaction in MANOVA: $p = 0.004$). According to the results of univariate ANOVAs (Table 3), females ran faster (both for average and maximum speed), and they performed fewer but longer runs, after oviposition (Figure 1). The interactions between the effects of gravidity and site of origin on maximum distance and average speed were significant (Table 3): when gravid, but not after oviposition, females from low-elevation origin ran significantly shorter distances and at a slower average speed than high-elevation females (Figure 1). Gravid low-elevation females ran at $83.4 \pm 3.8\%$ of their post-laying average speed, whereas gravid high-elevation females ran at $94.7 \pm 3.1\%$ of their post-laying average speed.

Table 3. General linear models for the data shown in Figure 1.

Predictor	Wilks' λ	Rao's F	df	p	Variable(s) significantly associated with predictor (univariate tests)
Model 1					
Gravidity	0.476	7.43	4, 27	<0.001	No. of runs**, Av. speed***, Max. speed***, Max. dist.**
Gravidity \times site	0.581	4.87	4, 27	0.004	Av. speed*, Max. dist.**
Model 2					
Gravidity	0.704	2.73	4, 26	0.051	Av. speed*, Max. speed**, Max. dist.**
Gravidity \times site	0.574	4.82	4, 26	0.005	Av. speed*, Max. dist.**
Temperature	0.904	0.69	4, 26	0.606	–
Model 3					
Gravidity	0.884	0.86	4, 26	0.503	–
Gravidity \times site	0.715	2.59	4, 26	0.060	Max. dist.**
Time to lay	0.922	0.55	4, 26	0.701	–
Model 4					
Gravidity	0.927	0.49	4, 25	0.740	–
Gravidity \times site	0.706	2.60	4, 25	0.061	Max. dist.**
Temperature	0.906	0.65	4, 25	0.632	–
Time to lay	0.924	0.52	4, 25	0.724	–

The first four columns correspond to multivariate MANOVAs (Model 1) or MANCOVAs (Models 2–4), whereas the last column indicates the dependent variables that were significantly associated with the corresponding predictor in univariate ANOVAs or ANCOVAs.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

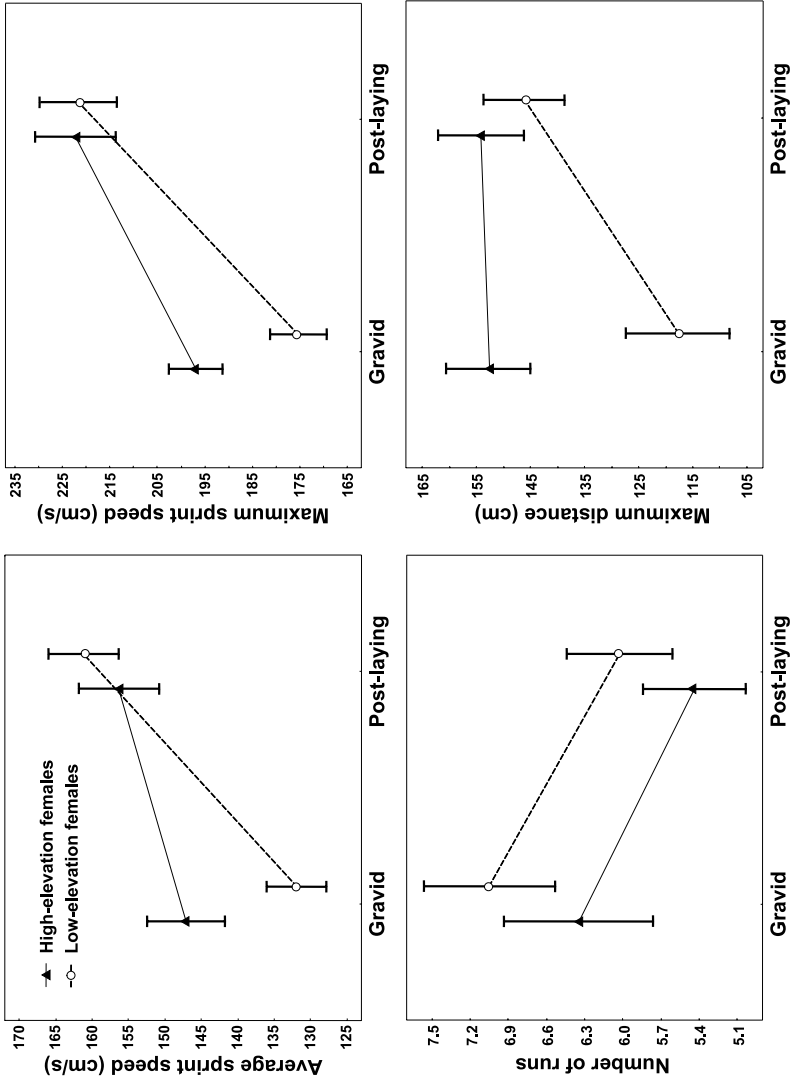


Figure 1. Variation of performance and behavioural traits (mean ± SE) in gravid and post-laying females from the high- and low-elevation sites.

Females of both populations had lower temperatures when gravid (high-elevation: $33.1 \pm 1.0^\circ\text{C}$, $N = 13$; low-elevation: $33.0 \pm 1.1^\circ\text{C}$, $N = 19$) than after oviposition (high-elevation: $34.1 \pm 0.9^\circ\text{C}$, $N = 13$; low-elevation: $34.3 \pm 1.0^\circ\text{C}$, $N = 19$; repeated measures ANOVA: $F_{1,30} = 31.10$, $p < 0.001$; gravidity \times site interaction: $F_{1,30} = 0.73$, $p = 0.398$). There were no between-population differences in body temperatures either before ($F_{1,30} = 0.07$, $p = 0.800$) or after laying ($F_{1,30} = 0.50$, $p = 0.484$). Temperature had no effect on performance or escape strategy in gravid females (all p values > 0.3), but it had a positive effect on both average running speed ($F_{1,29} = 6.23$, $p = 0.018$) and maximum sprint speed ($F_{1,29} = 7.31$, $p = 0.011$) after oviposition. However, neither maximum distance nor number of runs were affected by temperature in post-laying females (both p values > 0.4), suggesting that sprint capacity was more dependent on body temperature than escape strategy. A multivariate analysis (Model 2 MANCOVA in Table 3) showed that the effects of temperature and gravidity were partially confounded, because females had lower temperatures at the time of trial when gravid than after laying and they ran shorter distances at a lower speed; nevertheless, the differences between sites in the effects of gravidity on average speed and maximum distance ran (i.e., the gravidity \times site interactions) were unaffected by temperature.

However, between-sites differences were largely attributable to population disparity in mean time to lay at the moment of running (low-elevation: 3 ± 0.2 days, $N = 19$; high-elevation: 6 ± 0.5 days, $N = 13$; $F_{1,30} = 31.10$, $p < 0.001$) due to the earlier laying date of low-elevation females (mean laying date = 27 May ± 0.4 days, $N = 19$) relative to high-elevation ones (mean laying date = 31 May ± 0.8 days, $N = 13$; $F_{1,30} = 31.77$, $p < 0.001$). Thus, the gravidity \times site interaction was borderline significant in the repeated-measures MANCOVAs with mean time to lay (or mean time to lay plus temperature) as the covariate(s) (Table 3: Wilks' $\lambda = 0.715$, Rao's $F_{4,26} = 2.59$ and $p = 0.060$ for Model 3; and Wilks' $\lambda = 0.706$, Rao's $F_{4,25} = 2.60$ and $p = 0.061$ for Model 4), indicating that some of the results for individual dependent variables might reach significance. Accordingly, low-elevation females ran significantly shorter distances than high-elevation ones when gravid, but not after oviposition (Table 3: $p = 0.01$ in both univariate ANCOVAs). Stepwise multiple regressions (both forward and backward) confirmed that the effects of gravidity were more pronounced in the low- than in the high-elevation females for average speed ($F_{1,30} = 5.16$, $p = 0.031$) and maximum distance ($F_{1,30} = 9.06$, $p = 0.005$).

Field data

The mean distance to the nearest refuge (i.e., shrub in which a lizard could seek shelter) was larger at the low-elevation (127.6 ± 23.5 cm, $N = 50$) than at the high-elevation site (36.2 ± 6.3 cm, $N = 50$; $F_{1,98} = 14.15$, $p < 0.001$). Accordingly, space use and antipredatory behaviour of gravid females in the field differed significantly between sites. When first sighted, low-elevation females were found more frequently within refuges (9 of 11 gravid females) than high-elevation ones (2 of 7 gravid females; $\chi^2 = 5.1$, $p = 0.024$), despite the lower availability of refuges at low-elevation. The mean height above the ground of the perching sites of gravid females was higher at low (12.9 ± 2.3 cm) than at high elevation (5.0 ± 2.7 cm; $F_{1,16} = 4.80$, $p = 0.043$).

Discussion

Our data show that gravidity had significant effects on locomotor performance, escape strategy, and body temperature selection: when gravid, females escaped using a larger number of shorter runs, ran at lower speeds, and had lower body temperatures at the time of trial than after laying. Some of these effects were more pronounced in low-elevation females, which ran shorter distances than high-elevation ones when gravid, but not after egg laying. In this section, we try to frame these findings in their appropriate ecological context, combining them with observations of field behaviour which indicate that differences between both populations in antipredatory tactics could be associated with habitat structure.

Reduction in locomotor performance during pregnancy may be an important reproductive cost for females if it leads to an increased risk of mortality (Schwarzkopf & Shine, 1992; Miles et al., 2000; Olsson et al., 2000). Previous studies have suggested that reduced locomotor performance is a direct result of carrying the physical burden of the clutch (Shine, 2003; Zani et al., 2008), which in *P. algirus* can account for up to one fourth of the body mass of females (mean relative clutch mass = 0.25 ± 0.01 , with no significant differences between sites). Impaired locomotion in gravid females was observed in both populations, but their responses in lab running trials were not equivalent. This could be explained by differences in morphology (e.g., size or shape), physiology (e.g., thermoregulation), life histories (egg and clutch

size, extent of egg retention), or behaviour (different escape tactics evolved under natural conditions in association with different types of antipredator strategies).

Concerning differences in morphology, for instance in hind limb length (Losos & Sinervo, 1989; Losos, 1990), no significant differences were found for any of the traits examined. Therefore, the population-specific responses of gravid females cannot be explained solely by these factors. On the other hand, low-elevation females laid smaller clutches of larger eggs than high-elevation ones, i.e., the allocation of reproductive investment along the clutch size vs. egg size trade-off was different in the two populations. However, low-elevation females, despite bearing fewer eggs, experienced a more pronounced change towards shorter and slower runs. This is an unexpected finding, that might indicate different behavioural responses rather than differences in performance capacity, although we cannot discard the possibility that mean egg size could affect other morphological parameters associated with running performance such as stride length (Martín & Avery, 1998) or body curvature (Medger et al., 2008).

The slower speed of gravid females might also be related to their lower body temperatures, given the well-known thermal dependence of sprint speed in lizards (Huey & Stevenson, 1979; Huey, 1982; Hertz et al., 1983; Van Damme et al., 1989; Bauwens et al., 1995) and the fact that pregnant females should maximize reproductive benefits by shifting thermal preferences towards the levels optimal for embryonic development (e.g., Van Damme et al., 1992; Braña & Ji, 2007). However, our results showed that body temperatures did not differ between populations, which is consistent with the evolutionary rigidity of thermal biology in lacertid lizards (Van Damme et al., 1989, 1990; Díaz, 1997) and with previous reports of temperature selection by this species (see Díaz et al., 2006). As a consequence, thermal differences can not be responsible for the observed between-population differences in performance or behaviour. Body temperature influenced the running performance of post-laying females but not that of gravid ones, which suggests that females ran close to their maximal capabilities after oviposition, whereas their performance when gravid (especially in low-elevation lizards) may represent a behavioural strategy rather than reflect their actual locomotor capacity.

On average, low-elevation females laid their clutches earlier than high-elevation ones, which could be a consequence of an earlier start of the breeding season at low-elevation and/or longer egg retention by high-elevation

females. This interpretation is supported by the longer incubation time of the low-elevations eggs (Iraeta et al., 2006), which could reduce the overall period of locomotor impairment due to gravidity at the low-elevation site. In addition, the earlier laying date of low-elevation females was at least partly responsible of the observed between-populations difference in the effect of gravidity on average speed, because the mean time to lay at the moment of running was shorter in low-elevation than in high-elevation gravid females, and both mean egg mass and clutch mass increase steadily during gravidity. In fact, when controlling for the effects of laying date, the reduction of average speed in gravid females did not differ significantly between populations, but the shortening of the distance run was still more pronounced in low-elevation than in high-elevation females. This suggests that our results reflect differences in antipredatory behaviour rather than in locomotor capacity.

Such behavioural differences should be interpreted in the light of between-sites variation in habitat structure and associated predation risk. We are fully conscious that, because only two populations are compared, our reasoning inevitably involves the confounding of independent variables (see Garland & Adolph, 1994, for a detailed critique of the problem posed by two-species comparative studies), so that all environmental or genetic factors that distinguish both populations could account for the observed differences between them. Therefore, our discussion and conclusions must be tentative in this point. Nevertheless, it is well known that the escape responses of lizards are fine-tuned to microhabitat variation in vegetation (Irschick, 2003), and behavioural traits associated with predator evasion capacity may evolve rapidly at the population level in response to the amount of vegetation cover (Gifford et al., 2008). Consistently with this view, we found differences in habitat structure and habitat use, because refuge availability was lower, and percentage of time perching above ground (i.e., inside shelter-providing shrubs) was higher, at the lowland site. Also, it is important to note that predation risk was apparently higher at the low-elevation site, where lizards were easier to capture by humans (and perhaps other predators). Previous studies have shown that, in several lizard species, gravid females shift their escape tactics towards a longer application of crypsis (Vitt & Congdon, 1978; Bauwens & Thoen, 1981; Cooper et al., 1990), and our low-elevation females, by remaining close to or inside refuges (Díaz, 1992), managed to reduce drastically their conspicuousness, as deduced from the fact that in many occasions

they were detected only after we had spent a large amount of time searching for lizards inside vegetation patches. It should also be noted that at the high-elevation site, gravid females can bask on the ground without facing high risk of predation given the short average distance to the nearest available refuge. However, at the low-elevation site perching on top of the low shrub layer (and, as a consequence, increasing the mean height of perching sites) is probably the best way to combine basking opportunities and shelter-seeking, together with the selection of the appropriate compass directions around the perimeter of shrubs (east, southeast and south in the morning, vs. south, southwest and west in the afternoon; Díaz, 1992).

The shorter distance run by gravid lowland females in the laboratory may, thus, represent a behavioural response to minimize detectability. Such behavioural adjustments (shorter runs and more time basking above ground inside refuges) might inhibit selection for evolutionary shifts in the running performance of gravid females, thus, buffering the variation in selective pressures on gravid females imposed by environmental differences (Schulte et al., 2004 and see Huey et al., 2003 for a similar reasoning about the role of behavioural thermoregulation as an inertial factor in physiological evolution), especially if the probability that an animal will be predated depends primarily on whether or not it is seen by the predator, rather than on how quickly it can escape (Schwarzkopf & Shine, 1992). Our results support the view that, to understand the antipredator strategies evolved by gravid females of different populations, greater integration of laboratory studies of performance and behavioural/ecological studies is required (Arnold, 1983; Irschick & Garland, 2001).

Acknowledgements

This paper is a contribution to the projects CGL2004-01151/BOS and CGL2007-02744/BOS, funded by the Spanish Ministry of Education and Science. P.I. was funded by a PhD studentship from the Universidad Complutense de Madrid and C.M. was funded by a CSIC-El Ventorrillo grant. Permissions to capture lizards were provided by the Patrimonio Nacional (owner of El Pardo) and the Dirección General del Medio Natural of the Madrid region. Tobias Uller and two anonymous reviewers gave useful comments on a previous version of the manuscript.

References

- Arnold, E.N. (1987). Resource partition among lacertid lizards in southern Europe. — *J. Zool.* (B) 1: 739-782.

- Arnold, S.J. (1983). Morphology, performance and fitness. — *Am. Zool.* 23: 347-361.
- Bauwens, D., Garland, T., Castilla, A.M. & Van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. — *Evolution* 49: 848-863.
- Bauwens, D. & Thoen, C. (1981). Escape tactics and vulnerability to predation associated to reproduction in the lizard (*Lacerta vivipara*). — *J. Anim. Ecol.* 50: 733-743.
- Blouin-Demers, G. & Weatherhead, P.J. (2001). Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. — *Ecology* 82: 3025-3043.
- Braña, F. (1993). Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. — *Oikos* 66: 216-222.
- Braña, F. & Ji, X. (2007). The selective basis for increased egg retention: early incubation temperature determines hatchling phenotype in wall lizards (*Podarcis muralis*). — *Biol. J. Linn. Soc.* 92: 441-447.
- Cooper, W.E., Vitt, L.J., Hedes, R. & Huey, R.B. (1990). Locomotor impairment and defence in gravid lizards (*Eumeces laticeps*): behavioural shift in activity may offset cost of reproduction in an active forager. — *Behav. Ecol. Sociobiol.* 27: 153-157.
- Díaz, J.A. (1992). Choice of compass directions around shrub patches by the heliothermic lizard *Psammotromus algerus*. — *Herpetologica* 48: 293-300.
- Díaz, J.A. (1997). Ecological correlates of the thermal quality of an ectotherm's habitat: a comparison between two temperate lizard populations. — *Funct. Ecol.* 11: 79-89.
- Díaz, J.A. & Carrascal, L.M. (1991). Regional distribution of a Mediterranean lizard: influence of habitat cues and prey abundance. — *J. Biogeogr.* 18: 291-297.
- Díaz, J.A., Iraeta, P. & Monasterio, C. (2006). Seasonality provokes a shift of thermal preferences in a temperate lizard, but altitude does not. — *J. Therm. Biol.* 31: 237-242.
- Díaz, J.A., Pérez-Tris, J., Bauwens, D., Pérez-Aranda, D., Carbonell, R., Santos, T. & Tellería, J.L. (2007). Reproductive performance of a lacertid lizard at the core and the periphery of the species' range. — *Biol. J. Linn. Soc.* 92: 87-96.
- Díaz, J.A., Pérez-Tris, J., Tellería, J.L., Carbonell, R. & Santos, T. (2005). Reproductive investment of a lacertid lizard in fragmented habitat. — *Conserv. Biol.* 19: 1578-1585.
- Downes, S.J. & Shine, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? — *Ecology* 82: 1293-1303.
- Garland, T. & Adolph, S.C. (1994). Why not to do two-species comparative-studies — limitations on inferring adaptation. — *Phys. Zool.* 67: 797-828.
- Gifford, M.E., Herrel, A. & Mahler, D.L. (2008). The evolution of locomotor morphology, performance, and anti-predator behaviour among populations of *Leiocephalus* lizards from the Dominican Republic. — *Biol. J. Linn. Soc.* 93: 445-456.
- Hertz, P.E., Huey, R.B. & Nevo, E. (1983). Homage to Santa-Anita — thermal sensitivity of sprint speed in agamid lizards. — *Evolution* 37: 1075-1084.
- Huey, R.B. (1982). Temperature, physiology, and the ecology of reptiles. — In: *Biology of the Reptilia*, vol. 12 (Gans, C. & Pough, F.H., eds), Academic Press, London, p. 25-91.
- Huey, R.B., Hertz, P.E. & Sinervo, B. (2003). Behavioral drive versus behavioural inertia in evolution: a null model approach. — *Am. Nat.* 161: 357-366.
- Huey, R.B. & Stevenson, R.D. (1979). Integrating thermal physiology and ecology of ectotherms — discussion of approaches. — *Am. Zool.* 19: 357-366.
- Husack, J.F. (2006). Does speed help to survive? A test with collared lizard of different ages. — *Funct. Ecol.* 20: 174-176.

- Iraeta, P., Monasterio, C., Salvador, A. & Díaz, J.A. (2006). Mediterranean hatchling lizards grow faster at higher altitude: a reciprocal transplant experiment. — *Funct. Ecol.* 20: 865-872.
- Iraeta, P., Salvador, A. & Díaz, J.A. (2008). A reciprocal transplant study of activity, body size and winter survivorship in juvenile lizards from two sites at different altitude. — *Ecoscience* 15: 298-304.
- Irschick, D.J. (2003). Studying performance in nature: implications for fitness variation within populations. — *Integr. Comp. Biol.* 43: 36-47.
- Irschick, D.J. & Garland, T.D. (2001). Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. — *Annu. Rev. Ecol. Syst.* 32: 367-396.
- Irschick, D.J., Herrel, A.V., Vanhooydonck, B., Huyghe, K. & Van Damme, R. (2005). Locomotor compensation creates a mismatch between laboratory and field estimates of escape speed in lizards: a cautionary tale for performance-to-fitness studies. — *Evolution* 59: 1579-1587.
- Ji, X., Lin, L.H., Luo, L.G., Lu, H.L., Gao, J.F. & Han, J. (2006). Gestation temperature affects sexual phenotype, morphology, locomotor performance, and growth of neonatal brown forest skinks, *Sphenomorphus indicus*. — *Biol. J. Linn. Soc.* 88: 453-463.
- Ji, X., Lin, C.X., Lin, L.H., Qiu, Q.B. & Du, Y. (2007). Evolution of viviparity in warm-climate lizards: an experimental test of the maternal manipulation hypothesis. — *J. Evol. Biol.* 20: 1037-1045.
- Le Galliard, J.F., Le Bris, M. & Clobert, J. (2003). Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. — *Funct. Ecol.* 17: 877-885.
- Losos, J.B. (1990). The evolution of form and function: morphology and locomotor performance ability in West Indian *Anolis* lizards. — *Evolution* 44: 1189-1203.
- Losos, J.B., Creer, D.A. & Schulte, J.A. (2002). Cautionary comments on the measurement of maximum locomotor capabilities. — *J. Zool.* 258: 57-61.
- Losos, J.B. & Sinervo, B. (1989). The effects of morphology and perch diameter on sprint performance in *Anolis* lizards. — *J. Exp. Biol.* 145: 23-30.
- Lourdais, O., Heulin, B. & DeNardo, D.F. (2008). Thermoregulation during gravidity in the children's python (*Antaresia childreni*): a test of the preadaptation hypothesis for maternal thermophily in snakes. — *Biol. J. Linn. Soc.* 93: 499-508.
- Martín, J. & Avery, R.A. (1998). Effects of tail loss on the movement patterns of the lizard, *Psammotromus algirus*. — *Funct. Ecol.* 12: 794-802.
- Martín, J. & López, P. (1995). Influence of habitat structure on the escape tactics of the lizard *Psammotromus algirus*. — *Can. J. Zool.* 73: 129-132.
- Martín, J. & López, P. (2000). Fleeing to unsafe refuges: effects of conspicuousness and refuge safety on the escape decisions of the lizard *Psammotromus algirus*. — *Can. J. Zool.* 78: 265-270.
- Mathies, T. & Andrews, R.M. (1997). Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? — *Funct. Ecol.* 11: 498-507.
- Medger, K., Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the cape dwarf gecko, *Lygodactylus capensis*. — *Ethology* 114: 42-52.

- Miles, D.B., Sinervo, B. & Frankino, W.A. (2000). Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. — *Evolution* 54: 1386-1395.
- Olsson, M., Shine, R. & Bak-Olsson, E. (2000). Locomotor impairment of gravid lizards: is the burden physical or physiological? — *J. Evol. Biol.* 13: 263-268.
- Pérez-Tris, J., Díaz, J.A. & Tellería, J.L. (2004). Loss of body mass under predation risk: cost of antipredatory behaviour or adaptive fit-for-escape? — *Anim. Behav.* 67: 511-521.
- Reznick, D. (1985). Costs of reproduction: an evaluation of the empirical evidence. — *Oikos* 44: 25-267.
- Schulte II, J.A., Losos, J.B., Cruz, F.B. & Núñez, H. (2004). The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae: Liolaemini). — *J. Evol. Biol.* 17: 408-420.
- Schwarzkopf, L. & Shine, R. (1992). Costs of reproduction in lizards: escape tactics and susceptibility to predation. — *Behav. Ecol. Sociobiol.* 31: 17-25.
- Shine, R. (1980). 'Costs' of reproduction in reptiles. — *Oecologia* 46: 92-100.
- Shine, R. (2003). Effects of pregnancy on locomotor performance: an experimental study on lizards. — *Oecologia* 136: 450-456.
- Sinervo, B. (1999). Mechanistic analysis of natural selection and a refinement of Lack's and William's principles. — *Am. Nat.* 154: S26-S42.
- Sinervo, B. & DeNardo, D.F. (1996). Costs of Reproduction in the wild: path analysis of natural selection and experimental tests of causation. — *Evolution* 50: 1299-1313.
- Van Damme, R., Bauwens, D., Braña, F. & Verheyen, R.F. (1992). Incubation temperature differentially affects embryo survival, development and hatchling performance in the lizard *Podarcis muralis*. — *Herpetologica* 48: 220-228.
- Van Damme, R., Bauwens, D., Castilla, A.M. & Verheyen, R.F. (1989). Altitudinal variation of the thermal biology and running performance in the lizard *Podarcis tiliguerta*. — *Oecologia* 80: 516-524.
- Van Damme, R., Bauwens, D. & Verheyen, R.F. (1990). Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. — *Oikos* 57: 61-67.
- Veiga, J.P. & Salvador, A. (2001). Individual consistency in emergence date, a trait affecting mating success in the lizard *Psammodromus algirus*. — *Herpetologica* 57: 99-104.
- Vitt, L.J. & Congdon, J.D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. — *Am. Nat.* 112: 595-608.
- Williams, G.C. (1966). Natural selection, the cost of reproduction, and a refinement of Lack's principle. — *Am. Nat.* 100: 687-690.
- Zani, P.A., Neuhaus, R.A., Jones, T.D. & Milgrom, J.E. (2008). Effects of reproductive burden on endurance performance in side-blotched lizards (*Uta stansburiana*). — *J. Herpetol.* 42: 76-81.
-