

## **Dorsal pattern polymorphism in female Iberian wall lizards: differences in morphology, dorsal coloration, immune response and reproductive investment**

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Sex-specific color polymorphisms have been extensively documented in many different taxa. When polymorphism in color pattern is restricted to females the condition is known as female-limited pattern polymorphism (FPP) which has been less commonly addressed in vertebrates. FPP is present in several lizard species but most research on lizards has focused on carotenoid- and pteridine-based coloration but not on melanin-based polymorphisms. Here we focus on Iberian wall lizards, *Podarcis hispanicus*, where two female melanin-based dorsal patterns can be clearly distinguished: striped and reticulated-blotched. We indirectly tested the hypothesis that selection acts differentially among *P. hispanicus* female morphs to create alternative morph-specific phenotypic optima at different levels, by testing whether morphs differ in fitness proxies. We specifically examined whether the two female dorsal pattern morphs differed in adult morphology, dorsal coloration, immune response, reproductive investment and growth. We did not find a relationship between melanin-based coloration and hatchling growth and immune response, in spite that a correlation between these traits might be expected due to a pleiotropy in the melanocortin system. However, our results show that female dorsal morphs in *P. hispanicus* differ in terms of adult morphology, dorsal coloration and reproductive investment. Reticulated-blotched *P. hispanicus* females had deeper heads and longer femora, less melanin and more brownish coloration, and had larger and heavier hatchlings than striped females.

**ADDITIONAL KEYWORDS:** growth – Lacertidae – immune response – reproductive investment – female-limited polymorphism – common garden – melanin-based coloration

## INTRODUCTION

The study of natural variation has long fascinated evolutionary biologists and attempts to account for this variation were major contributors to the formulations of Darwin's idea of evolution (Russell & Bauer, 2005). In contrast to continuous traits, discrete polymorphisms provide tractable systems because they are easy to categorize and, hence, simplify the study of evolutionary dynamics in the wild (Calsbeek, Bonvini & Cox, 2010a). Polymorphism is defined as the occurrence of two or more distinct phenotypic forms within a single population of a species (Forsman and Shine, 1995). Polymorphic traits can be found at the species, population, ontogenetic or sex level (Forsman & Shine, 1995).

When only females are polymorphic, while males are monomorphic and do not exhibit the same range of variation in pattern as females, the condition is known as female-limited pattern polymorphism (FPP; Stamps & Gon, 1983). Sex-specific color polymorphisms associated with different life-histories have been extensively documented in many different taxa, such as insects, isopods, molluscs and vertebrates (Oliveira *et al.*, 2008). Although the interest in FPP has increased substantially (Svensson, 2009; Cox & Calsbeek, 2012; Ortega, López & Martín, 2014) a bias towards insect studies exists, whereas vertebrates have been less studied (Paemelaere, Guyer, & Dobson, 2011). Color polymorphism is a common phenomenon in lizards (e.g. Forsman & Shine, 1995; Sinervo *et al.*, 2000; Vercken *et al.*, 2007), a group with the most compelling examples of alternative reproductive strategies linked to different morphs. In the common lizard, *Zootoca vivipara*, females can be found in three ventral color morphs (i.e. yellow, orange and intermediate), which are correlated with several fitness-related traits such as clutch size, hatching success and litter sex ratio (Vercken *et al.*, 2007). In a similar fashion, female side blotched lizards (*Uta stansburiana*) present two distinct throat morphs (yellow and orange) that display alternative reproductive strategies, differing in clutch size, egg mass, and immune function (Sinervo, Svensson & Comendant, 2000), which is linked to complex physiological and behavioural syndromes (Sinervo, 2001; Svensson *et al.*, 2001). Thus, most research on lizards has focused on carotenoid- and pteridine-based coloration but not on melanin-based polymorphisms (e.g. Sinervo & Zamudio, 2001; Vercken *et al.*, 2007; Galeotti *et al.*, 2013). On the other hand, several *Anolis* species present FPP where females within a population generally show two or three variations in melanin-based mid-dorsal patterns (i.e. a vertebral stripe, a diamond pattern or a dull pattern that resembles males; see Calsbeek, Bonneaud & Smith, 2008; Paemelaere *et al.*, 2011), whereas males are usually the less patterned sex and are rarely striped. In addition, in the common lizard, *Z. vivipara*, the frequency of individuals with a reticulated dorsal pattern is higher in males than in females (Lepetz *et al.*, 2009). Thus, a general trend for non-striped male patterns may exist in some lizards, suggesting distinct selective pressures between male and female dorsal patterns. However, the evolutionary processes underlying female-limited polymorphism are not well understood.

A melanin-based dorsal pattern polymorphism also occurs in the Iberian wall lizard, *Podarcis hispanicus*, a small lacertid lizard distributed throughout the Iberian Peninsula and North-Western Africa (Guillaume, 1987; Salvador and Carretero, 2014). In the Guadarrama Mountains (Central Spain) populations, two female dorsal patterns can be clearly distinguished (i.e. striped and reticulated-blotched), while males are all reticulated-blotched (Ortega *et al.*, 2014). A previous research concluded that hatchling antipredatory behaviour is influenced by dorsal patterns, independently of sex (Ortega *et al.*, 2014). Despite the lack of morphological size differences (i.e. body size, head size and femoral length) between dorsal morphs at hatching, we do not know whether adult morphology differs between female dorsal pattern morphs. Thus, since antipredatory behavior likely has a physiological rather than morphological basis in this lizard species (Ortega *et al.*,

2014), we could expect a similar morphology between the two adult female dorsal pattern morphs.

On the other hand, in vertebrates, darker individuals are predicted to have better anti-inflammatory immune responses than lighter ones due to a pleiotropy in the melanocortin system (Ducrest, Keller & Roulin, 2008). Moreover, melanin-based coloration traits have been proposed to function as honest indicators of health status in a similar fashion as carotenoid-based traits (Stoehr, 2006; Galván & Alonso-Alvarez, 2008; Metcalfe & Alonso-Alvarez, 2010). However, this relationship has rarely been explored in lizards. Vroonen, Vervust & Van Damme (2013) found that melanin pigments signal aspects of immune capacity in male common lizards *Z. vivipara*. However, studies of differences in immune response between dorsal morphs of female *Anolis sagrei* have yielded contradicting results (Calsbeek *et al.*, 2008; Cox & Calsbeek, 2011). Preliminary observations suggested that striped female *P. hispanicus* might be darker (i.e. more melanized) than reticulated-blotched ones (J. Ortega personal observ.), and hence, we predicted a better immune response of more melanic females, in general, and striped females, in particular. In addition, melanocortins are known to affect growth in vertebrates (Ducrest *et al.*, 2008). Nonetheless, it is difficult to predict the sign of covariation, which could be species and condition dependent in wild populations (Roulin *et al.*, 2008). Thus, in this study we also investigated the influence of melanization on lizard growth, a relationship that has never been explored in lizards. A negative correlation between growth and locomotor performance has been described in some vertebrates (Kolok and Oris, 1995; Arendt, 1997; Arendt, 2003). Hence, in accordance with the fast growers sprint lower hypothesis (Álvarez and Metcalfe, 2007; Shaun, Marras and McKenzie, 2014), we predicted that, as reticulated-blotched lizards have higher sprint speed (Ortega *et al.*, 2014), they would show a slower growth compared to striped females.

Trade-offs between reproduction and survival are important determinants of life-history characteristics (Roff, 1992; Stearns, 1992). Locomotor performance is an ecologically relevant trait that potentially influences survival by affecting the ability to escape from predators (Christian & Tracy, 1981; Webb, 1986). Across reproductive cycles females experience many physical and physiological changes that can be costly (Johnson *et al.*, 2010). For example, pregnancy and reproductive burden are known to impair locomotion and increase predation risk by decreasing sprint speed (Sinervo, Hedges & Adolph, 1991; Olsson, Shine & Bak-Olsson, 2000; Plaut, 2002; Shine, 2003), endurance (Miles, Sinervo & Frankino, 2000; Zani *et al.*, 2008) and acceleration (Rodewald & Foster, 1998; Scales & Butler, 2007). Differences in reproductive investment between dorsal pattern morphs have been examined in two lizard species but either differences were not found (in *Anolis sagrei*; see Cox & Calsbeek, 2011) or were mediated by the larger size of one morph (in *Z. vivipara*; see Lepetz *et al.*, 2009). In the case of *A. sagrei*, differences between dorsal morphs in escape behavior and sprint speed were not found (Les *et al.*, 2014). However, the fact that hatchling *P. hispanicus* female morphs show marked escape behavior differences (Ortega *et al.*, 2014) suggests the existence of contrasting trade-offs between antipredatory behavior and reproduction between morphs. Ghalambor, Reznick and Walker (2004) examined the degree to which differences in reproductive allocation reduce burst swimming performance in the Trinidadian guppy. They found that maximum and average velocity and the cumulative distance travelled declined as pregnancy progressed, and the rate of this decline was higher in guppies with higher reproductive allocation. The cost of gravidity might, thus, differ between *P. hispanicus* female morphs and, hence, we predicted that reticulated-blotched females could sacrifice reproductive investment to maintain higher maximal and mean sprint speed. To test this hypothesis, we specifically examined, for each female morph, clutch size, egg morphology and hatchling morphology.

Although FPP seems to be more common in nature than previously thought (Kunte 2009; Svensson et al 2009), most of the research has focused on males, interpreting female morphs as a byproduct of selection on males (Fisher 1930; Lande 1987). In keeping with this bias, few examples are available among vertebrates, and much emphasis has been placed on conspicuous coloration. Thus the study of non-conspicuous morphs in *P. hispanicus* females from a life history perspective that not only focus on reproductive investment may help us to understand the coexistence of alternative phenotypes within populations which is one of the most exciting and major challenges of the evolutionary theory. In the present work, we indirectly tested the hypothesis that selection acts differentially among *P. hispanicus* female morphs to create alternative morph-specific phenotypic optima at different levels, by testing whether morphs differ in fitness proxies. We specifically examined adult morphology, dorsal coloration, immune response and reproductive investment differences between the two female dorsal pattern morphs. We also examined morphology and growth of hatchlings born from the two female morphs.

## MATERIAL AND METHODS

### STUDY SITES

Male and gravid female *P. hispanicus* lizards were captured by noosing at two nearby populations in the Guadarrama Mountains (Central Spain) in April-May 2011, 2012 and 2013. These populations are separated 6 km by air at different altitudinal ranges. The lowland locality is 'La Dehesa de la Golondrina', an oak forest near Cercedilla village (40°44'N, 04°02'W; 1,250 m altitude). The highland population is located at the upper part of 'Fuenfría' Valley (40°47'N, 04°03'W; 1,750 m altitude). For details on interpopulational differences in environmental characteristics and morphology of adult lizards see Gabriot, López & Martín (2013a). It is worth noting that the *P. hispanicus* populations within this geographical area have just been named as a separate species within this complex (*P. g. guadarramae*; see Geniez *et al.*, 2014). Adult striped females are more abundant than reticulated-blotched females in both populations (Fuenfría: 66.1 % ; Golondrina: 60 %), but there are not significant interpopulational differences in proportion of morphs (Ortega *et al.*, 2014).

Captured lizards were immediately transported to "El Ventorrillo" field station facilities about 5 km away from the capture localities. Lizards were kept in individual plastic terraria located inside two climatic chambers (Ibercex V-450-D walk-in chambers; ASL S.A., Madrid, Spain) where ambient temperature (diurnal = 21°C; nocturnal = 15°C) and photoperiod (12 h:12 h, light: dark) were easily controlled automatically. A 50 W halogen lamp was suspended over one end of each terrarium providing a diurnal temperature gradient (21-45 °C) within the terrarium, which allows thermoregulation of lizards within the preferred temperature range of this species (34.4 °C; Bauwens *et al.*, 1995). In addition, a mercury vapor bulb (Exoterra Solar Glow 125 W) provided ultraviolet radiation. Adult lizards were returned to their capture sites in late June, and juveniles released to their mother capture sites in November prior to winter.

### MORPHOLOGICAL MEASUREMENTS

We measured body size of adult ( $N = 127$ ) and hatchling ( $N = 218$ ) lizards using a rule (snout-vent length, 'SVL', to the nearest 1 mm) and hatchling 'body weigh' using a digital scale (to the nearest 0.01 g). We used a digital caliper to measure (to the nearest 0.05 mm) the following morphological variables: 'head length' was the distance between the tip of the snout and the posterior side of the parietal scales; 'head width' was the greatest distance between the external sides of the parietal scales; 'head height' was the greatest distance from the highest portion of the head to the bottom of the lower jaw. 'Femoral

length' was the mean distance from the hip to the knee measured in both hindlimbs. A magnifying glass was used to count the number of femoral pores in the ventral side of left and right thighs in adults. For statistical analyses, hatchling morphology was clutch averaged to avoid pseudoreplication ( $N = 83$  clutches). We used general linear models (GLMs) to analyze whether these morphological variables (log-transformed) differed between female morphs (fixed effect). Due to the divergent morphology between the two studied populations (Gabirot *et al.*, 2013a; Ortega *et al.*, 2015), we also included population of origin as a random factor. As our preliminary analyses did not show any significant population x morph interaction (all  $P$ 's  $> 0.085$ ) or year effect (all  $P$ 's  $> 0.532$ ) we excluded them in these and further analyses.

#### DORSAL COLORATION

Eumelanic and pheomelanic traits are generally associated with specific colours, the former being responsible for black and grey colours and the latter for yellowish, reddish, and brown ones (Toral, Figuerola & Negro, 2008; Galván & Møller, 2011). Although reptiles were thought to produce only eumelanin (Ito & Wakamatsu, 2003), recently Roulin, Mafli & Wakamatsu (2013) were able to demonstrate their capacity to produce pheomelanin too. However, we will use the terms brownish and yellowish coloration as pheomelanin production in lizards has not been confirmed yet. On the other hand, we assume that patches of black coloration are produced by melanin, in accordance with most research on lizards (Morrison, Rand & Frost-Mason, 1995; Rosenblum *et al.*, 2010; Sacchi *et al.*, 2012; Vroonen *et al.*, 2013). Both striped and reticulated-blotched *P. hispanicus* show dorsal patterns characterized by three distinct colour categories, the first corresponding to the darkest part of the pattern (black), and the yellowish coloration, usually present in minor proportion around the striped or blotched area, and the darker brownish coloration, which is clearly recognizable in the most central part of the back. Our *a priori* classification of morphs was based on the distribution pattern of the different dorsal colors. To quantify percentages of different types of coloration we followed the procedure adopted by Galeotti *et al.* (2011) for yellow cheek-patches of the Hermann's tortoise. We took a dorsal picture of adult females ( $N = 42$ ) and hatchlings ( $N = 45$ ) from the two morphs. Photos were taken the day after capture for the adults, and 5 days after hatching for the hatchlings, but all under the same standardized indoor conditions (distance, light and exposure set constant for all pictures), using a digital camera (Canon EOS 350 D) with 3456 x 2304 pixels of resolution and 16384 colours per channel. Each picture was then transferred to the computer and analyzed with Adobe Photoshop CS2. For each photo, we selected the area between the outermost edge of yellowish bands, which corresponded to striped or blotched surface. The three different types of coloration were measured (in pixels) using the 'magic wand' option (tolerance 32) basing on RGB values as reference, in order to obtain an accurate estimate of the percentage of each class of colors by respect to the total of the selected surface. The repeatability (Lessels & Boag, 1987) of the measures, calculated for each of the three color categories, as assessed on two replicates of each picture, was very high (black coloration:  $r = 0.99$ ,  $F_{86,87} = 1725$ ,  $P < 0.001$ ; yellowish coloration:  $r = 0.99$ ,  $F_{86,87} = 655$ ,  $P < 0.001$ ; brownish coloration:  $r = 0.99$ ,  $F_{86,87} = 1659$ ,  $P < 0.001$ ), indicating that the measurement error was negligible. To normalize the data, all coloration percentages were converted to proportions and logit transformed ( $\log(p/[1-p])$ ; where  $p$  is proportion in a 0 to 1 scale) (Warton & Hui, 2011).

The relationship between melanin-based coloration, adult female immune response (see below) and hatchling growth was examined with general regression models (GRM). Melanin (independent variable) was included in general regression models (GRM), one for each growth variable and one for adult female immune response (as dependent variables).

## IMMUNE RESPONSE

We measured the inflammatory response in 41 adult females of both morphs (31 striped, 10 reticulated). We used a delayed-type hypersensitivity test, the phytohaemagglutinin (PHA) injection test (Svensson, Sinervo & Comendant, 2001; Belliure, Smith & Sorci, 2004). This test is considered a multifaceted index of cutaneous immune activity that is initiated by T-cells and involves both innate and adaptive components of the immune system (Martin *et al.*, 2006; Salaberria *et al.*, 2013). We used this test because we were interested in using swelling as a standardized index of immunocompetence, independently of the type of immune cells involved in the response. We marked a point with permanent ink on the right foot pad, and measured the thickness at this point with a pressure sensitive spessimeter (to the nearest 0.01 mm) (Mitutoyo, Aurora, IL, USA) to standardize pressure during measurements. Then, we injected 0.02 mg of PHA dissolved in 0.01 mL of phosphate buffered saline (PBS) water in the foot pad. Lizards were released in their terraria, and after 24 h we measured again the foot pad thickness at the marked point. The immune response was calculated as the difference between pre- and postinjection measures (Smits *et al.*, 1999; Belliure *et al.*, 2004). The only appreciable effect of the PHA injection was a slight swelling of the skin, due to the immune response, which disappeared after 48 h. None of the lizards showed any signs of stress or pain during these tests. Assays took place after parturition (Richard *et al.*, 2012), since the endocrine profile is known to affect immunity (Sorci, Møller & Boulinier, 1997; Roitt, Brostoff & Male, 1998; Reid, Arcese & Keller, 2003). Moreover, the costly nature of the activation of the immune system posits the existence of trade-offs between reproduction and immune response, both processes known to compete for limited resources (Nordling *et al.*, 1998; Imonen, Taarna & Hasselquist, 2000; Cox & Calsbeek, 2011). We used general linear models (GLMs) to test for immune response differences (log-transformed) between populations and female dorsal morphs.

## EGGS AND HATCHLING HUSBANDRY

During the 2011 breeding season, we performed a common garden experiment where eggs were incubated and newborns raised under standardized indoor conditions. Females laid eggs that were individually placed in 60-mL closed plastic cups filled with 10 g of moistened perlite (10 g perlite:10 g water) and transferred to an incubator at 27.5 °C (IRE-160; 94 × 60 × 60 cm; Raypa, Barcelona). Eggs were randomly distributed in the incubator and shelves rotated each week to control for possible position effects (Telemeco *et al.* 2010). We measured clutch size (number of eggs), egg size (length and width, using a digital caliper to the nearest 0.01 mm) and egg weight (using a digital scale to the nearest 0.01 g). Egg volume was calculated using the equation for volume of an ellipsoid  $V=(4/3)\pi ab^2$ , where  $a$  is half the longest axis and  $b$  is half the shortest axis (Mayhew, 1963). Clutch volume was calculated as the sum of the volume of all the eggs of each clutch. We calculated relative clutch size of each female (i.e. clutch size adjusted for female SVL) using the residuals of the regression of log-clutch size on log-female SVL. Hatchling lizards were kept in a climatic chamber under identical environmental conditions than adults (see above), but fed smaller prey (Ortega *et al.*, 2015). Hatchling cages were rotated along shelf rows every three days and among shelves each week to control for position effects (Telemeco *et al.*, 2010). All hatchlings (100%) did not change dorsal pattern during approximately 5 months after hatching. Sex was assessed, and confirmed several times during the common garden experiment, by the highly developed femoral pores in males compared to females. We tested for differences between observed sex ratios (proportion of sons to total offspring produced by females of each morph) and a theoretical one of 1:1 using  $\chi^2$  tests in a subset of 2011 females ( $N = 28$ ) for which the sex of hatchlings could be determined.

We focused our analysis on first clutches as they potentially reflect the field conditions (e.g. food availability) experienced by females in the wild before being captured, minimizing captivity effects in our experiment. To test for differences between morphs in mean egg size, clutch size, clutch mass, clutch volume and hatchling morphology, we analyzed these variables as dependent variables in general linear models (GLMs), with the population of origin as a random effect. We also ran similar GLMs but with ‘female SVL’, ‘clutch size’, ‘egg mass’ or ‘hatchling SVL’ as covariates when necessary. We analyzed hatchling growth rates in a subset of sexed hatchlings captive born in 2011 ( $N = 49$ ) with a nested design in GLM using the statistical package Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). The size-specific (SVL), mass-specific, head and femoral length growth between the hatching date and 130 days of age were expressed as the proportionate increase in size or mass [ $\ln(\text{size at the end}/\text{size at the beginning})/(\text{end date} - \text{initial date})$ ], measured in units of  $\text{days}^{-1}$  (Iraeta et al., 2012). We summarized sex and dorsal polymorphism in a single variable (‘sex/dorsal morph’; as in Ortega *et al.*, 2014; hereafter sex/morph for brevity) with three levels: striped female, reticulated-blotched female and male (males are monomorphic with a reticulated-blotched pattern). The mixed model tested the significance of clutch (female identity as a random factor, nested in female morph) to control for possible familiar effects, female morph and hatchling sex/morph (fixed factors) on hatchling growth. We used Satterthwaite’s method of denominator synthesis and required leaving the random effects independent of the fixed effects (Searle, Casella & McCulloch, 1992). As fractions of variance components were used to synthesize error terms for significance testing, the degrees of freedom for the denominator mean square can be fractional rather than integer values (Iraeta *et al.*, 2012). All variables were log-transformed prior analysis to meet the requirements of parametric tests. All statistical analyses, here and elsewhere, were conducted in Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA).

## RESULTS

### ADULT MORPHOLOGY

Overall, inter-population differences were significant for all morphological variables, highland females being larger than lowland ones. However, independently of inter-population differences in body size, reticulated-blotched females had significant larger head height and femoral length than striped females (see Table 1). However, these differences disappeared after controlling for body size (morph effect in GLMs with SVL head height:  $F_{1,123} = 2.98$ ,  $P = 0.087$ ; femoral length:  $F_{1,123} = 1.18$ ,  $P = 0.280$ ).

### DORSAL COLORATION

There were significant differences between female morphs and age categories in the percentages of melanin (GLM: morph:  $F_{1,60} = 7.90$ ,  $P = 0.007$ ; population:  $F_{1,60} = 0.62$ ,  $P = 0.433$ ; age:  $F_{1,60} = 8.40$ ,  $P = 0.005$ ; morph x age:  $F_{1,60} = 0.10$ ,  $P = 0.756$ ) (Fig. 1). Thus, striped females were darker (i.e. had more black coloration) than reticulated-blotched females, and hatchlings were darker than adults independently of the dorsal pattern.

Also, there were significant differences between female morphs and age categories in the percentages of brownish coloration (GLM: morph:  $F_{1,60} = 8.53$ ,  $P = 0.005$ ; population:  $F_{1,60} = 1.10$ ,  $P = 0.300$ ; age:  $F_{1,60} = 10.19$ ,  $P = 0.002$ ; morph x age:  $F_{1,60} = 0.02$ ,  $P = 0.893$ ) (Fig. 1). Thus reticulated-blotched females had more brownish coloration than striped females. In addition, hatchlings had less brownish coloration than adults independently of the dorsal pattern.

Yellowish coloration did not differ between female morphs or age categories (GLM: morph:  $F_{1,60} = 0.01$ ,  $P = 0.967$ ; population:  $F_{1,60} = 0.53$ ,  $P = 0.469$ ; age:  $F_{1,60} = 1.29$ ,  $P = 0.260$ ; morph x age:  $F_{1,60} = 0.03$ ,  $P = 0.876$ ) (Fig. 1).

#### IMMUNE RESPONSE

We did not find any significant difference in the PHA skin swelling immune response between female dorsal morphs or between populations (immune response: reticulated-blotched =  $0.19 \pm 0.02$  mm; striped =  $0.17 \pm 0.01$  mm; GLM: morph:  $F_{1,38} = 1.57$ ,  $P = 0.218$ ; population:  $F_{1,38} = 0.90$ ,  $P = 0.349$ ). This lack of differences between female morphs remained not significant even after excluding population as a factor (GLM: morph:  $F_{1,39} = 1.76$ ,  $P = 0.193$ ).

Our results did not support a relationship between immune response and melanin based coloration. The GRM examining the potential relationship between melanin and the immune response was not significant ( $R^2 = 0.02$ ,  $F_{1,39} = 0.89$ ,  $P = 0.351$ ).

#### REPRODUCTIVE INVESTMENT

Mean clutch size, clutch volume and clutch weigh did not significantly differ between reticulated-blotched and striped females (see Table 2). However, highland females laid larger, heavier and more voluminous clutches than lowland ones. Egg mass and egg volume did not significantly differ between populations or female morphs. Neither the incubation period was significantly different between populations or female morphs (Table 2). The sex ratio of offspring produced by females did not significantly differ from a theoretical sex ratio of 1:1 in either reticulated-blotched (3 males vs. 8 females;  $\chi^2_1 = 0.79$ ,  $P = 0.37$ ) or striped females (22 males vs. 25 females;  $\chi^2_1 = 0.17$ ,  $P = 0.68$ ). Hatchlings born from reticulated-blotched females were significantly larger and heavier than hatchlings born from striped females (Table 2), even after controlling for egg mass (GLMs with egg mass as a covariant: egg mass:  $F_{1,80} = 101.75$ ,  $P < 0.001$ ; morph:  $F_{1,80} = 4.27$ ,  $P = 0.042$ ; population:  $F_{1,80} = 0.51$ ,  $P = 0.477$ ) or clutch size (GLMs with clutch size as a covariant: clutch size:  $F_{1,80} = 2.63$ ,  $P = 0.109$ ; morph:  $F_{1,80} = 8.44$ ,  $P = 0.005$ ; population:  $F_{1,80} = 5.0$ ,  $P = 0.028$ ). Body condition did not differ between populations or female dorsal morphs. Hatchlings born from reticulated-blotched females also had significantly longer and wider, but not deeper, heads and longer femora than hatchlings born from striped females (Table 2), but this was the result of their absolute larger body size (GLMs with hatchling SVL as a covariate: hatchling SVL:  $P$ 's  $< 0.001$  in all cases; morph:  $P$ 's  $> 0.720$  in all cases; population:  $P$ 's  $> 0.142$  in all cases).

#### HATCHLING GROWTH

A mixed model GLM of the different growth variables (Table 3), with clutch (female identity as a random factor) nested in female morph (fixed factor) and sex/morph (fixed effect) did not detect significant differences between offspring from both female morphs in mass-specific growth, size specific growth, head growth or femoral growth, nor between sex/morph of hatchlings. A significant effect of clutch was found for mass-specific growth, size-specific growth and head length growth (see Table 3). This clutch effect disappeared after controlling for relative clutch size and egg weigh in the case of size-specific growth (mixed model GLM: clutch size:  $F_{1,17.00} = 0.87$ ,  $P = 0.364$ , clutch:  $F_{23,17.00} = 1.81$ ,  $P = 0.106$ ; mixed model GLM: egg weigh:  $F_{1,17.00} = 0.17$ ,  $P = 0.685$ , clutch:  $F_{23,17.00} = 1.78$ ,  $P = 0.112$ ) and after controlling for egg weigh in the case of mass-specific growth (mixed model GLM: egg weigh:  $F_{1,17.00} = 0.05$ ,  $P = 0.824$ , clutch:  $F_{23,17.00} = 2.09$ ,  $P = 0.061$ ) and relative clutch size in the case of head length (mixed model GLM: clutch size:  $F_{1,17.00} = 1.06$ ,  $P = 0.318$ , clutch:  $F_{23,17.00} = 1.63$ ,  $P = 0.152$ ).



## RELATIONSHIP BETWEEN GROWTH AND MELANIN BASED COLORATION

None of the GRMs examining the potential relationships between melanin and growth (mass-specific growth, size specific growth, head growth or femoral growth) was significant (all  $P$ 's > 0.264). Thus we cannot support the existence of a relationship between growth and melanin based coloration.

## DISCUSSION

Overall, our results show that female dorsal morphs in *P. hispanicus* differ in terms of adult morphology, melanin-based coloration and reproductive investment. Reticulated-blotched *P. hispanicus* females had deeper heads and longer femora, less melanin and more brownish dorsal coloration, and had larger and heavier hatchlings than striped females.

Reticulated-blotched and striped females showed contrasting melanin based coloration differences. Thus, female morphs not only differ in melanin distribution, which determines the dorsal pattern, but also in terms of the amount of melanization. Striped females, independently of their age, have a higher percentage of melanin (black) and a lower percentage of brownish dorsal coloration than reticulated-blotched ones, while they do not differ in percentages of yellowish coloration. This pattern is also found in *Z. vivipara* where the hypothesis of a more efficient thermoregulation has been raised to explain why striped females are darker (Lepetz *et al.*, 2009) as darker reptiles are able to warm faster and maintain higher body temperatures for longer (Majerus, 1998; Bittner *et al.*, 2002; Gabirot *et al.*, 2013b).

In a similar fashion, we have detected an ontogenetic trajectory in dorsal melanin-based coloration. Overall, hatchlings had a higher percentage of melanin, and a lower percentage of brownish coloration than adults. Ontogenetic variation in coloration is a common phenomenon in reptiles but the adaptive significance of color change that is not directly related to reproduction remains poorly understood (Booth, 1990). As darker reptiles have several thermoregulation advantages (Majerus, 1998; Bittner *et al.*, 2002), we can hypothesize that thermoregulation constraints imposed by a small body size (i.e., low thermal inertia, fast heating rates coupled with fast cooling rates; Carrascal *et al.*, 1992; Martín & López, 2003; Gabirot *et al.*, 2013b) may be, at least partially, responsible for the darker hatchling dorsal coloration in *P. hispanicus* and other lacertids like *Z. vivipara* (Vroonen *et al.*, 2013). However, we cannot dismiss the possible role of crypsis in hatchlings and striped females as it was not the focus of this research.

Reticulated-blotched females had larger head height and femoral length than striped females but these differences disappeared after controlling for body size. These results point out that differences between morphs are likely to be in general size, although body size differences were only marginally significant (see Table 1). It is worth noting that the error measurement for size is higher than for the other morphological measurements, reducing the probability of finding significant results. Males from these populations are characterized by deeper heads and longer femora (Ortega *et al.*, 2015). Thus, reticulated blotched females, at least regarding these variables, are more similar to males than striped females. This finding raises the possibility that reticulated blotched lizards, independently of sex, might share similarities in some physiological traits.

We also found that hatchlings born from reticulated-blotched females were larger and heavier than hatchlings born from striped females. This relationship remained significant even after controlling for mean egg size and clutch size, as a trade-off between egg and hatchling size, and between progeny size and number may exist in lizards (Svensson, Sinervo & Comendant, 2001; Vercken *et al.*, 2007). These morphological differences are independent of egg mass and, hence, do not depend on the amount of

resources allocated to the eggs. However, the observed morphological pattern is thus more likely to result from genetically based differences in physiology, as female dorsal morph differences in escape behaviour also do not have an underlying morphological basis (Ortega *et al.*, 2014). A contrasting physiology (e.g. endocrine profile) between dorsal morphs during egg production might be responsible for this pattern. As we did not find sex ratio differences between morphs, the effect of maternal color morph on hatchling morphology may reflect either maternal environmental or genetic effects (Platenkamp & Shaw, 1993). Overall, the reproductive investment differences between *P. hispanicus* morphs are in sharp contrast with research focusing on dorsal pattern polymorphism in other lizard species (Lepetz *et al.*, 2009; Cox & Calsbeek, 2011). Correspondences between color polymorphism and alternative reproductive tactics often occur because coloration functions as a visual signal that mediates social interactions between morphs (Vercken *et al.*, 2007). However, most evidence shows that dorsal coloration is generally presumed to be an adaptation for crypsis or thermoregulation in diurnal reptiles (Norris, 1965; Kettlewell, 1973; Jackson *et al.*, 1976). Nonetheless, although odoriferous cues are more important, there is evidence for the role of coloration to elicit courtship and deter male aggressive response at long range in *P. hispanicus* (López & Martín, 2001; López, Martín & Cuadrado, 2002). As opposed to *Anolis sagrei*, the best known example of dorsal female-limited polymorphism in lizards (Calsbeek *et al.*, 2008; Cox & Calsbeek, 2011, Les *et al.*, 2014), hatchling *P. hispanicus* dorsal pattern morphs differ in sprint speed and escape behavior (Ortega *et al.*, 2014). This fact prompted us to hypothesize the existence of trade-offs between antipredatory behaviour and reproduction (i.e. egg and clutch size) because the cost of gravidity may differ between morphs. However, our results do not support this hypothesis.

Some studies suggest that fast growth comes at the expense of locomotor performance (Álvarez & Metcalfe, 2007; Shaun *et al.*, 2014). However, we did not find support for the trade-off between growth and locomotor performance in this species as we did not detect growth differences between the two female morphs, which differ in terms of locomotor performance (Ortega *et al.*, 2014). In a similar fashion, the morph of the mother did not influence the growth of its offspring, suggesting an absence of maternal effects affecting lizard growth. In spite that melanin-based coloration is known to affect the growth of some vertebrates (Ducrest *et al.*, 2008), the proportion of dorsal melanin did not influence lizard growth.

Increased immune function can lead to trade-offs with other traits that are important for fitness (e.g. sexual ornamentation; Verhulst, Dieleman & Parmentier, 1999). Thus the optimal investment in immunity will depend not only on the risk of infection but also on the competing needs of other life-history traits (Sinervo & Calsbeek, 2003). Differential investment in life-history traits may lead to correlational selection favouring alternative life-history strategies with different fitness optima and this is thought to play a key role in the evolution of polymorphism (Calsbeek *et al.*, 2008). Nevertheless, we did not detect differences in immune response between female dorsal morphs nor correlations between immune response and melanin-based coloration. This is in sharp contrast with the work of Vroonen *et al.*, (2013) where male, but not female, *Z. vivipara* with more melanic ventral coloration mounted a stronger PHA-induced immune response. However we did not examine this relationship in males and the black ventral patterning in *P. hispanicus* is very scarce, with just a few small black dots localized in the gular or the ventro-lateral areas (Salvador & Carretero, 2014).

In summary, we have shown how *P. hispanicus* morphs have contrasted reproductive strategies. Hatchlings born from reticulated-blotched females were larger and heavier than those born from striped ones, which suggest the role of maternal effects mediating offspring phenotype differences between the two morphs. We also have shown

that morphs and age classes differ in terms of melanin based and brownish dorsal coloration. Adult striped and hatchling females were darker than reticulated-blotched adult females and hatchlings, respectively. Moreover, adult female morphs had different morphology as reticulated-blotched females had deeper heads and longer femora than striped ones. Our results also suggest that this female-limited polymorphism is not maintained by selective pressures related to immune response. We also neglect the existence of a relationship between melanin-based coloration and hatchling growth and immune response. Although the *P. hispanicus* species complex poses some problems such as morph heritability estimation, its wide geographical distribution and the high variation in morph frequencies both within and between lineages offer a great opportunity to shed light on the evolution and maintenance of female-limited polymorphisms.

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#### REFERENCES

- Álvarez D, Metcalfe NB. 2007.** The tradeoff between catch-up growth and escape speed: variation between habitats in the cost of compensation. *Oikos* **116**: 1144-1151.
- Andres JA, Sanchez-Guillen RA, Rivera AC. 2000.** Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. *Evolution* **54**: 2156–2161.
- Shaun SK, Marras S, McKenzie DJ. 2014.** Fast growers sprint slower: effects of food deprivation and re-feeding on sprint swimming performance in individual juvenile European sea bass. *The Journal of Experimental Biology* **217**: 859-65
- Arendt JD. 1997.** Adaptive intrinsic growth rates: An integration across taxa. *The Quarterly Review of Biology* **72**:149–177.
- Arendt JD. 2003.** Reduced burst speed is a cost of rapid growth in anuran tadpoles: problems of auto-correlation and inferences about growth rates. *Functional Ecology* **17**: 328-334.
- Bauwens D, Garland T, Jr, Castilla AM, Van Damme R. 1995.** Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. *Evolution* **49**: 848-863.
- Belliure J, Smith L, Sorci G. 2004.** Effect of testosterone on T cell-mediated immunity in two species of Mediterranean lacertid lizards. *Journal of Experimental Zoology A* **301**: 411–418.
- Bittner TD, King RB, Kerfin JM. 2002.** Effects of body size and melanism on the thermal biology of garter snakes (*Thamnophis sirtalis*). *Copeia* **2002**: 477–482.
- Booth CL. 1990.** Evolutionary significance of ontogenic colour change in animals. *Biological Journal of the Linnean Society* **40**: 125–163.
- Calsbeek R, Bonneaud C, Smith TB. 2008.** Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. *Journal of Animal Ecology* **77**: 103–109.

- Calsbeek R, Bonvini L, Cox RM. 2010.** Geographic variation, frequency-dependent selection, and the maintenance of a female-limited polymorphism. *Evolution* **64**: 116–125.
- Calsbeek B, Hasselquist D, Clobert J. 2010.** Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour morphs. *Journal of Evolutionary Biology* **23**: 1138–1147.
- Carrascal LM, López P, Martín J, Salvador A. 1992.** Basking and antipredator behaviour in a high altitude lizard: implications of heat-exchange rate. *Ethology* **92**: 143–154.
- Christian KA, Tracy CR. 1981.** The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia* **49**: 218–223.
- Cox RM, Calsbeek R. 2011.** An experimental test for alternative reproductive strategies underlying a female-limited polymorphism. *Journal of Evolutionary Biology* **24**: 343–353.
- Ducrest A, Keller L, Roulin A. 2008.** Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology and Evolution* **23**: 502–510.
- Fisher RA. 1930.** *The genetical theory of natural selection*. Oxford: Clarendon Press.
- Forsman A, Shine R. 1995.** The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata*. *Biological Journal of the Linnean Society* **55**: 273–291.
- Gabirot M, López P, Martín J. 2013.** Female mate choice based on pheromone content may inhibit reproductive isolation between distinct populations of Iberian wall lizards. *Current Zoology* **59**: 210–220.
- Gabirot M, Balleri A, López P, Martín J. 2013.** Variations in thermal biology between two morphologically distinct populations of Iberian wall lizards inhabiting different environments. *Annales Zoologici Fennici* **50**: 225–236.
- Ghalambor CK, Reznick DN, Walker JA. 2004.** Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* **164**: 38–50
- Galeotti P, Sacchi R, Pellitteri-Rosa D, Fasola M. 2011.** The yellow cheek-patches of the Hermann's tortoise: sexual dimorphism and relationship with body condition. *Italian Journal of Zoology* **78**: 464–470.
- Galeotti P, Sacchi R, Pellitteri-Rosa D, Bellati A, Cocca W, Gentilli A, Scali S, Fasola M. 2013.** Colour polymorphism and alternative breeding strategies: effects of parent's colour morph on fitness traits in the Common wall lizard. *Evolutionary Biology* **40**: 385–394.
- Galván I, Alonso-Álvarez C. 2008.** An intracellular antioxidant determines the expression of a melanin-based signal in a bird. *PlosOne* **3**: e3335.
- Galván I, Møller AP. 2011.** Brain size and the expression of pheomelanin-based colour in birds. *Journal of Evolutionary Biology* **24**: 999–1006.
- Geniez P, Sá-Sousa P, Guillaume CP, Cluchier A, Crochet PA. 2014.** Systematics of the podarcis hispanicus complex (sauria, lacertidae) III: valid nomina of the western and central iberian forms. *Zootaxa* **3794**: 1–51.
- Guillaume CP. 1987.** Les petits Lacertidés du Bassin Méditerranéen Occidental (Genera *Podarcis* et *Archeolacerta* essentiellement). PhD Thesis, Languedoc, Montpellier: University of Science and Technology.
- Ilmonen P, Taarna T, Hasselquist D. 2000.** Experimentally activated immune defense in female pied flycatchers results in reduced breeding success *Proceedings of the Royal Society B* **267**: 665–670.

- Iraeta P, Salvador A, Díaz JA. 2012.** Life-history traits of two Mediterranean lizard populations: a possible example of countergradient covariation. *Oecologia* **172**: 167–176.
- Ito S, Wakamatsu K. 2003.** Quantitative analysis of eumelanin and pheomelanin in humans, mice and other animals: a comparative review. *Pigment Cell Research* **16**: 523–531.
- Jackson JF, Ingram W, Campbell HW. 1976.** The dorsal pigmentation pattern of snakes as an antipredator strategy: a multivariate approach. *American Naturalist* **110**: 1029–1053.
- Johnson M, Caton JL, Cohen RE, Vandecar JR, Wade J. 2010.** The burden of motherhood: The effect of reproductive load on female lizard locomotor, foraging, and social behavior. *Ethology* **116**: 1217–1225.
- Kettlewell B. 1973.** *The evolution of melanism*. Oxford: Clarendon Press.
- Kolok AS, Oris TJ. 1995.** The relationship between specific growth rate and swimming performance in male fathead minnows (*Pimephales promelas*). *Canadian Journal of Zoology* **73**:2165–2167.
- Kunte K. 2009.** Female-limited mimetic polymorphism: a review of theories and a critique of sexual selection as balancing selection. *Animal Behaviour* **78**:1029–1036.
- Lande R. 1987.** *Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences*. In: Bradbury JW, Andersson MB, eds. *Sexual selection: testing the alternatives*. London: Wiley, 83-95.
- Lepetz V, Massot M, Chaine AS, Clobert J. 2009.** Climate warming and the evolution of morphotypes in a reptile. *Global Change Biology* **15**: 454–466.
- Les AM, Gifford ME, Parmerlee JS Jr., Powell R. 2014.** Do Polymorphic Female Brown Anoles (*Anolis sagrei*) Differ in Sprint Speed or Escape Behavior?. *Herpetologica* **70**: 47-55.
- Lessels CM, Boag PT. 1987.** Unrepeatable repeatabilities: A common mistake. *Auk* **104**: 116–121.
- López P, Martín J. 2001.** Pheromonal recognition of females takes precedence over the chromatic cue in male iberian wall lizards, *Podarcis hispanica*. *Ethology* **107**: 901–912.
- López P, Martín J, Cuadrado M. 2002.** Pheromone mediated intrasexual aggression in male lizards, *Podarcis hispanicus*. *Aggressive Behavior* **28**: 154–163.
- Majerus MEN. 1998.** *Melanism: evolution in action*. Oxford, U.K: Oxford University Press.
- Martin LB, Han P, Lewittes J, Kuhlman JR, Klasing KC, Wikelski M. 2006.** Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoeological technique. *Functional Ecology* **20**: 290–299.
- Martín J, López P. 2003.** Ontogenetic variation in antipredatory behavior of Iberian-rock lizards (*Lacerta monticola*): effects of body-size-dependent thermal-exchange rates and costs of refuge use. *Canadian Journal of Zoology* **81**: 1131–1137.
- Mayhew WW. 1963.** Reproduction in the granite spiny lizard, *Sceloporus orcutti*. *Copeia* **1963**: 144–152.
- Metcalfe NB, Alonso-Alvarez C. 2010.** Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology* **24**: 984–996.
- Miles DB, Sinervo B, Frankino WA. 2000.** Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution* **54**: 1386–1395.

- Morrison RL, Rand MS, Frost-Mason SK. 1995.** Cellular basis of color differences in three morphs of the lizard *Sceloporus undulatus erythrocheilus*. *Copeia* **1995**: 397-408.
- Nordling D, Andersson M, Zohari S, Gustafsson L. 1998.** Reproductive effort reduces specific immune response and parasite resistance. *Proceedings of the Royal Society B* **265**: 1291–1298.
- Norris KS. 1965.** Color adaptation in desert reptiles and its thermal relationships. In: W. W. Milstead, ed. *Lizard Ecology: A Symposium*. University of Missouri Press, Columbia, Missouri. 162–226.
- Oliveira RF, Taborsky M, Brockmann HJ. 2008.** *Alternative reproductive tactics: an integrative approach*. Cambridge: Cambridge University Press.
- Olsson M, Shine R, Bak-Olsson E. 2000.** Locomotor impairment of gravid lizards: is the burden physical or physiological? *Journal of Evolutionary Ecology* **13**: 263–268.
- Olsson M, Healey M, Wapstra E, Schwartz T, Lebas N, Uller T. 2007.** Mating system variation and morph fluctuations in a polymorphic lizard. *Molecular Ecology* **16**: 5307–5315.
- Ortega J, López P, Martín J. 2014.** Conspicuous blue tails, dorsal pattern morphs and scape behavior in hatchling Iberian wall lizards (*Podarcis hispanicus*). *Biological Journal of the Linnean Society* **113**: 1094–1106.
- Ortega J, López P, Martín J. 2015.** Altitudinally divergent adult phenotypes in Iberian wall lizards are not driven by egg differences nor hatchling growth rates. *Oecologia* **177**: 357–366.
- Paemelaere EAD, Guyer C, Dobson FS. 2011.** A phylogenetic framework for the evolution of female polymorphism in anoles. *Biological Journal of the Linnean Society* **104**: 303–317.
- Rosenblum EB, Rompler H, Schoneberg T, Hoekstra HE. 2010.** Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 2113–2117.
- Salvador A, Carretero MA. 2014.** *Podarcis hispanicus* (Steindachner, 1870). In: Salvador A, coord. *Fauna Iberica, Vol. 10. Reptiles*, 2<sup>a</sup> ed. Madrid: Museo Nacional de Ciencias Naturales, C.S.I.C., 537-556
- Platenkamp GAJ, Shaw RG. 1993.** Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. *Evolution* **47**: 540–555.
- Plaut I. 2002.** Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Functional Ecology* **16**: 290–295.
- Reguera S, Zamora-camacho FJ, Moreno-rueda G. 2014.** The lizard *Psammmodromus algirus* (Squamata: Lacertidae) is darker at high altitudes. *Biological Journal of the Linnean Society* **112**: 132–141.
- Reid JM, Arcese P, Keller LF. 2003.** Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and inter-generational effects. *Proceedings of the Royal Society B* **270**: 2151–2157.
- Richard M, Massot M, Clobert J, Meylan S. 2012.** Litter quality and inflammatory response are dependent on mating strategy in a reptile. *Oecologia* **170**: 39–46.
- Rodewald AD, Foster SA. 1998.** Effects of gravidity on habitat use and antipredator behaviour in three-spined sticklebacks. *Journal of Fish Biology* **52**: 973–984.
- Roff DA. 1992.** *The evolution of life histories: theory and analysis*. New York: Chapman and Hall.
- Roitt I, Brostoff J, Male D. 1998.** *Immunology, 5th ed*. London: Mosby.

- Roulin A, Gasparini J, Bize P. 2008.** Melanin-based coloration signal strategies to cope with poor and rich environments. *Behavioral Ecology and Sociobiology* **62**: 507–519.
- Roulin A, Mafli A, Wakamatsu K. 2013.** Reptiles produce pheomelanin : evidence in the Eastern Hermann's tortoise (*Eurotestudo boettgeri*). *Journal of Herpetology* **47**: 258-261.
- Salaberria C, Muriel J, de Luna M, Gil D, Puerta M. 2013.** The PHA test as an indicator of phagocytic activity in a passerine bird. *PloS One* **8**:e84108
- Sacchi R, PellitteriRosa D, Bellati A, Scali S, Tronconi D, Galeotti P, Fasola M. 2012.** Biological correlates of two dorsal color pattern types in the common Wall lizard, *Podarcis muralis* (Laurenti, 1768). *Herpetozoa* **25**: 3–11.
- Scales J, Butler M. 2007.** Are powerful females powerful enough? Acceleration in gravid green iguanas (*Iguana iguana*). *Integrative and Comparative Biology* **47**: 285–294.
- Searle SR, Casella G, McCullock CE. 1992.** *Variance components*. Wiley, New York
- Shine R. 2003.** Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia* **136**: 450–456.
- Sinervo B. 2001.** Runaway social games, genetic cycles driven by alternative male and female strategies, and the origin of morphs. *Genetica* **112–113**: 417–434.
- Sinervo B, Calsbeek R. 2003.** Physiological epistasis, ontogenetic conflict and natural selection on physiology and life history. *Integrative and Comparative Biology* **43**: 419–430.
- Sinervo B, Hedges R, Adolph SC. 1991.** Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: Variation among populations. *Journal of Experimental Biology* **155**: 323-336.
- Sinervo B, Zamudio, KR. 2001.** The evolution of alternative reproductive strategies: fitness differential, heritability, and genetic correlation between the sexes. *The Journal of Heredity* **92**: 198–205.
- Sinervo B, Svensson E, Comendant T. 2000.** Density cycles and an offspring quantity and quality game driven by natural selection. *Nature* **406**: 985–988.
- Smits JE, Bortolotti GR, Tella JL. 1999.** Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13**: 567–572.
- Sorci G, Møller AP, Boulinier T. 1997.** Genetics of host-parasite interactions. *Trends in Ecology and Evolution* **12**: 196–200.
- Stamps JA, Gon SM. 1983.** Sex-biased pattern variation in the prey of birds. *Annual Review of Ecology and Systematics* **14**: 231–253.
- Stearns SC. 1992.** *The evolution of life histories*. Oxford: Oxford University Press.
- Stoehr AM. 2006.** Costly melanin ornaments: the importance of taxon? *Functional Ecology* **20**: 276–281.
- Svensson E, Sinervo B, Comendant T. 2001.** Density-dependent competition and selection on immune function in genetic lizard morphs. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 12561–12565.
- Svensson EI, Abbott JK, Gosden TP, Coreau A. 2009.** Female polymorphisms, sexual conflict and limits to speciation processes in animals. *Evolutionary Ecology* **23**:93–108.
- Telemeco R, Radder R, Baird T, Shine R. 2010.** Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biological Journal of the Linnean Society* **100**: 642–655.
- Toral GM, Figuerola J, Negro JJ. 2008.** Multiple ways to become red: pigment identification in red feathers using spectrometry. *Comparative Biochemistry and Physiology B* **150**: 147–152.

- Vercken E, Massot M, Sinervo B, Clobert J. 2007.** Colour variation and alternative reproductive strategies in females of the common lizard *Lacerta vivipara*. *Journal of Evolutionary Biology* **20**: 221–232.
- Verhulst S, Dieleman SJ, Parmentier HK. 1999.** A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 4478–4481.
- Vroonen J, Vervust B, Van Damme R. 2013.** Melanin-based colouration as a potential indicator of male quality in the lizard *Zootoca vivipara* (Squamata: Lacertidae). *Amphibia-Reptilia* **34**: 539–549.
- Warner DA, Shine R. 2009.** Maternal and environmental effects on offspring phenotypes in an oviparous lizard: do field data corroborate laboratory data? *Oecologia* **161**: 209–220.
- Warton DI, Hui FK. 2011.** The arcsine is asinine: the analysis of proportions in ecology. *Ecology* **92**: 3–10.
- Webb PW. 1986.** Locomotion and predator-prey relationships. In: Lauder GV, Feder ME, eds. *Predator-Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates*. Chicago: University of Chicago Press, 24–41.
- Zani PA, Neuhaus RA, Jones TD, Milgrom JE. 2008.** Effects of reproductive burden on endurance performance in side-blotched lizards (*Uta stansburiana*). *Journal of Herpetology* **42**: 76–81.



**Table 1.** Mean ( $\pm 1$  SE) values for morphological variables of adult female *Podarcis hispanicus* lizards of two dorsal morphs (reticulated-blotched vs. striped) in highland and lowland populations. Results of GLMs testing the effects of female morph (fixed factor) and population (random factor) on the morphological variables are shown. Significant p-values are marked in bold.

	Highland		Lowland		GLMs			
	Reticulated-blotched ( <i>N</i> = 21)	Striped ( <i>N</i> = 41)	Reticulated-blotched ( <i>N</i> = 26)	Striped ( <i>N</i> = 40)	Female morph		Population	
					<i>F</i> <sub>1,124</sub>	<i>P</i>	<i>F</i> <sub>1,124</sub>	<i>P</i>
SVL (cm)	6.6 $\pm$ 0.1	6.5 $\pm$ 0.1	6.0 $\pm$ 0.1	5.7 $\pm$ 0.1	3.34	0.070	45.47	< <b>0.001</b>
Head length (mm)	12.0 $\pm$ 0.2	11.7 $\pm$ 0.1	10.9 $\pm$ 0.1	10.6 $\pm$ 0.1	3.56	0.061	89.90	< <b>0.001</b>
Head width (mm)	7.5 $\pm$ 0.1	7.4 $\pm$ 0.1	6.9 $\pm$ 0.1	6.7 $\pm$ 0.1	3.59	0.060	70.25	< <b>0.001</b>
Head height (mm)	5.1 $\pm$ 0.1	5.0 $\pm$ 0.1	4.5 $\pm$ 0.1	4.4 $\pm$ 0.1	6.42	<b>0.013</b>	121.81	< <b>0.001</b>
Femoral pores (n)	17.6 $\pm$ 0.3	17.5 $\pm$ 0.2	18.1 $\pm$ 0.2	18.2 $\pm$ 0.2	7.00	0.946	7.33	<b>0.008</b>
Femoral length (mm)	9.8 $\pm$ 0.2	9.6 $\pm$ 0.1	8.6 $\pm$ 0.1	8.3 $\pm$ 0.1	4.10	<b>0.045</b>	114.69	< <b>0.001</b>

**Table 2.** Mean ( $\pm 1$  SE) values and results, clutch averaged, of GLMs ( $F$ ,  $P$ ) testing the effect of female dorsal pattern (fixed factor) and population (random factor) on clutch and hatchling characteristics in offspring from reticulated-blotched and striped female *Podarcis hispanicus* ( $n$  = number of clutches). Significant values are marked in bold.

	Reticulated- blotched ( $N = 31$ )	Striped ( $N = 53$ )	Female morph		Population	
			$F$ 1,80	$P$	$F$ 1,80	$P$
<b>Clutch:</b>						
Clutch size (N)	3.0 $\pm$ 0.1	3.0 $\pm$ 0.1	0.94	0.336	17.09	< <b>0.001</b>
Clutch volume (mm <sup>3</sup> )	671.6 $\pm$ 51.5	650.8 $\pm$ 39.8	0.94	0.335	13.83	< <b>0.001</b>
Clutch weight (g)	0.91 $\pm$ 0.05	0.86 $\pm$ 0.04	3.38	0.070	21.45	< <b>0.001</b>
Egg mass (g)	0.30 $\pm$ 0.01	0.28 $\pm$ 0.01	3.08	0.083	2.62	0.109
Egg volume (mm <sup>3</sup> )	214.0 $\pm$ 11.3	213.5 $\pm$ 8.7	0.23	0.635	2.11	0.150
Incubation period (days)	46.2 $\pm$ 0.9	46.0 $\pm$ 0.7	0.01	0.999	0.0	0.852
<b>Hatchlings:</b>						
SVL (cm)	3.05 $\pm$ 0.04	2.89 $\pm$ 0.03	9.0	<b>0.004</b>	0.0	0.885
Body mass (g)	0.41 $\pm$ 0.01	0.38 $\pm$ 0.01	7.39	<b>0.008</b>	2.85	0.093
Body condition (g cm <sup>-1</sup> * 100)	0.5 $\pm$ 0.4	-0.3 $\pm$ 0.3	1.92	0.170	2.66	0.107
Head length (mm)	7.20 $\pm$ 0.05	7.07 $\pm$ 0.04	5.0	<b>0.028</b>	0.2	0.632
Head width (mm)	4.16 $\pm$ 0.04	4.04 $\pm$ 0.03	5.70	<b>0.019</b>	0.4	0.545
Head height (mm)	2.95 $\pm$ 0.04	2.89 $\pm$ 0.03	3.05	0.085	5.93	<b>0.017</b>
Femoral length (mm)	4.57 $\pm$ 0.06	4.35 $\pm$ 0.47	6.80	<b>0.011</b>	0.59	0.443

**Table 3.** Mean ( $\pm 1$  SE) growth rates ( $\text{days}^{-1} \times 10^{-4}$ ) and the effect of clutch, female dorsal morph, hatchling sex/morph and female morph x hatchling sex/morph interaction (mixed model GLM) on growth for six morphological variables of hatchlings of *Podarcis hispanicus* lizards reared under a common garden experiment.

	Clutch		Female morph		Hatchling sex/morph		Female morph x hatchling sex/morph	
	$F_{23,18.00}$	$P$	$F$	$P$	$F_{2,18.00}$	$P$	$F_{1,18.00}$	$P$
Size-specific growth	2.26	0.041	$F_{1,29.05} = 0.47$	0.479	0.52	0.605	1.97	0.177
Mass-specific growth	2.38	0.032	$F_{1,28.76} = 0.01$	0.989	0.04	0.960	0.56	0.463
Head length growth	2.18	0.048	$F_{1,29.26} = 1.87$	0.182	0.64	0.538	0.04	0.836
Head width growth	1.06	0.455	$F_{1,34.63} = 0.54$	0.468	0.69	0.515	0.04	0.836
Head height growth	1.03	0.481	$F_{1,34.90} = 1.19$	0.282	0.16	0.852	0.61	0.444
Femoral length growth	1.60	0.155	$F_{1,31.26} = 2.54$	0.121	0.12	0.888	0.24	0.937

**Figure 1.** Mean ( $\pm 1$  SE) values for dorsal coloration traits in hatchling and adult female *P. hispanicus* from the two female morphs.

