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

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Sex-specific colour polymorphisms have been extensively documented in many different taxa. When polymorphism in colour 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, although most research on lizards has focused on carotenoid- and pteridine-based coloration and not on melanin-based polymorphisms. In the present study, 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 investigating 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, despite a correlation between these traits possibly being expected as a result of 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 also had larger and heavier hatchlings than striped females. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **00**, 000–000.

ADDITIONAL KEYWORDS: common garden – female-limited – growth – Lacertidae – 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). By 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, 2010). Polymorphism is defined as the occurrence of two or more distinct phenotypic forms within a single population of a species (Forsman & Shine, 1995). Polymorphic traits can be found at the species, population, ontogenetic or sex level (Forsman & Shine, 1995).

When only females are polymorphic, whereas 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 colour polymorphisms associated with different life-histories have been extensively documented in many different taxa, such as insects, isopods, molluscs, and vertebrates (Oliveira, Taborsky & Brockmann, 2008). Although the interest in FPP has increased substantially (Svensson *et al.*, 2009; Cox & Calsbeek, 2011; Ortega, López & Martín, 2014), a bias towards insect studies exists, and vertebrates have received less attention (Paemelaere, Guyer & Dobson, 2011).

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Colour polymorphism is a common phenomenon in lizards (Forsman & Shine, 1995; Sinervo, Svensson & Comendant, 2000; Vercken *et al.*, 2007), comprising 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 colour 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 *et al.*, 2000), which are linked to complex physiological and behavioural syndromes (Sinervo, 2001; Svensson, Sinervo & Comendant, 2001). Thus, most research on lizards has focused on carotenoid- and pteridine-based coloration but not on melanin-based polymorphisms (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; 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 & Carretero, 2014). In the populations inhabiting Guadarrama Mountains (Central Spain), two female dorsal patterns can be clearly distinguished (i.e. striped and reticulated-blotched), whereas males are all reticulated-blotched (Ortega *et al.*, 2014). 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, because antipredatory behaviour 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-inflamatory immune responses than lighter ones as a result of 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 fashion similar to carotenoid-based traits (Stoehr, 2006; Galván & Alonso-Álvarez, 2008; Metcalfe & Alonso-Alvarez, 2010). However, this relationship has rarely been explored in lizards. Vroonen, Vervust & Van Damme (2013) reported that melanin pigments signal aspects of immune capacity in male common lizards *Z. vivipara*. 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, pers. 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, Gasparini & Bize, 2008). Thus, in the present 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 & Oris, 1995; Arendt, 1997, 2003). Hence, in accordance with the fast growers sprint lower hypothesis (Álvarez & Metcalfe, 2007; Shaun, Marras & McKenzie, 2014), we predicted that, as reticulated-blotched lizards have a 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; however, either differences were not found (*Anolis sagrei*; Cox & Calsbeek, 2011) or were mediated by the larger size of one morph (*Z. vivipara*; Lepetz *et al.*, 2009). In the case of *A. sagrei*, differences between dorsal morphs in escape behaviour and sprint speed were not found (Les *et al.*, 2014). However, the fact that hatchling *P. hispanicus* female morphs show marked escape behaviour differences (Ortega *et al.*, 2014) suggests the existence of contrasting trade-offs between antipredatory behaviour and reproduction between morphs. Ghalambor, Reznick & Walker (2004) examined the degree to which differences in reproductive allocation reduce burst swimming performance in the Trinidanian guppy and found that maximum and average velocity and the cumulative distance travelled declined as pregnancy progressed, and also that 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 appears to be more common in nature than previously assumed (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 nonconspicuous morphs in *P. hispanicus* females from a life history perspective that also focuses 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 study, 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 and May 2011, 2012 and 2013. These populations are separated, 6 km apart 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 a.s.l.). The highland population is located at the upper part of ‘Fuenfría’ Valley (40°47′N, 04°03′W; 1750 m a.s.l.). Details on interpopulational differences in environmental characteristics and morphology of adult lizards are provided in Gabirot, López & Martín (2013a). It is worth noting that the *P. hispanicus* populations in this geographical area have just been named as a separate species within this complex (*Podarcis guadarramae guadarramae*; Geniez *et al.*, 2014). Adult striped females are more abundant than reticulated-blotched females in both populations (Fuenfría: 66.1%; Golondrina: 60%), although there are no significant interpopulational differences in the proportion of morphs (Ortega *et al.*, 2014).

Captured lizards were immediately transported to ‘El Ventorrillo’ field station facilities approximately 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 : 12 h light/dark cycle) were easily controlled. 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 vapour 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 weight’ using a digital scale (to the nearest 0.01 g). We used a digital caliper to measure (to the nearest 0.05 mm) several 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). As a result of the divergent morphology between the two studied populations (Gabirot *et al.*, 2013a; Ortega, López & Martín, 2015), we also included population of origin as a random factor. Because our preliminary analyses did not show any significant population × morph interaction (all $P > 0.085$) or year effect (all $P > 0.532$), 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 assumed to produce only eumelanin (Ito & Wakamatsu, 2003), recently Roulin, Mafli & Wakamatsu (2013) were able to demonstrate their capacity to produce pheomelanin too. However, we use the terms brownish and yellowish coloration because pheomelanin production in lizards has not yet been confirmed. 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 clasification of morphs was based on the distribution pattern of the different dorsal colours. 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 captured a dorsal image of adult females ($N = 42$) and hatchlings ($N = 45$) from the two morphs. Photographs were taken the day after capture the adults, and 5 days after hatching for hatchlings, although all were captured under the same standardized indoor conditions (distance, light and exposure set constant for all images), using a digital camera (Canon EOS 350 D) with 3456 × 2304 pixels of resolution and 16 384 colours per channel. Each image was then transferred to the computer and analyzed with PHOTOSHOP CS2 (Adobe). For each photograph, 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, aiming to obtain an accurate estimate of the percentage of each class of colours by respect to the total of the selected surface. The repeatability (Lessels & Boag, 1987) of the measures calculated for each of the three colour categories, as assessed on two replicates of each image, 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 0–1 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 the GRMs: 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 *et al.*, 2001; Belliure, Smith & Sorci, 2004). This test is considered as a multifaceted index of cutaneous immune activity 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) to standardize pressure during measurements. Then, we injected 0.02 mg of PHA dissolved in 0.01 mL of phosphate-buffered saline 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, Bortolotti & Tella, 1999; Belliure *et al.*, 2004). The only appreciable effect of the PHA injection was a slight swelling of the skin as a result of 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) because 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, which are both processes known to compete for limited resources (Nordling *et al.*, 1998; Ilmonen, Taarna & Hasselquist, 2000; Cox & Calsbeek, 2011). We used 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). 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 $V = (4/3)\pi\alpha b^2$, where α 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 3 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-squared 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 because 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 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, version 8.0 (StatSoft Inc.). 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 (size at the end/size at the beginning)/(end date − initial date)], measured in units of days^{-1} (Iraeta, Salvador & Díaz, 2012). We summarized sex and dorsal polymorphism in a single variable ('sex/dorsal morph'; *sensu* 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 & McCullock, 1992). Because 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 were conducted in STATISTICA, version 8.0 (StatSoft Inc).

RESULTS

ADULT MORPHOLOGY

Overall, interpopulational differences were significant for all morphological variables, with highland females being larger than lowland ones. However, independently of interpopulational differences in body size, reticulated-blotched females had significant larger head height and femoral length than striped females (Table 1). 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 × 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.

There were also 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 × 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 × 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 ± 0.02 mm; striped = 0.17 ± 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 was still 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 differ significantly between reticulated-blotched and striped females (Table 2). However, highland females laid larger, heavier and more voluminous clutches than lowland ones. Egg mass and egg volume did not differ significantly between populations or female morphs. The incubation period was not significantly different between populations or female morphs (Table 2). The sex ratio of offspring produced by females did not differ significantly from a theoretical sex ratio of 1 : 1 in either reticulated-blotched (three males vs. eight 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:

Table 1. Mean ± SE values for morphological variables of adult female *Podarcis hispanicus* lizards of two dorsal morphs (reticulated-blotched vs. striped) in highland and lowland populations

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

Results of general linear models (GLMs) testing the effects of female morph (fixed factor) and population (random factor) on the morphological variables are shown. Significant values are marked in bold.

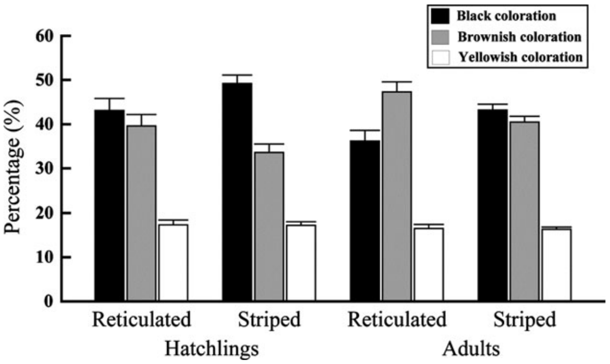

Figure 1. Mean ± SE values for dorsal coloration traits in hatchling and adult female *Podarcis hispanicus* from the two female morphs.

$F_{1,80} = 101.75$, $P < 0.001$; 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), although this was the result of their absolute larger body size (GLMs with hatchling SVL as a covariate: hatchling SVL: $P < 0.001$ in all cases; morph: $P > 0.720$ in all cases; population: $P > 0.142$ 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 (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 $P > 0.264$). 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 differ not only 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, whereas they do not differ in percentages of yellowish coloration. This pattern is also found in *Z. vivipara* where the hypothesis of a more efficient thermorregulation has been raised to explain why striped females are darker (Lepetz *et al.*, 2009) because darker reptiles are able to warm faster and maintain higher body temperatures for longer (Majerus, 1998; Bittner, King & Kerfin, 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, although the adaptive significance of colour change that is not directly related to reproduction remains poorly understood (Booth, 1990). Because 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

Table 2. Mean ± 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)

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

GLMs, general linear models. Significant values are marked in bold.

Table 3. Mean ± SE growth rates (day^{-1} × 10^{-4}) and the effect of clutch, female dorsal morph, hatchling sex/morph and female morph × hatchling sex/morph interaction (mixed model general linear model) 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 × 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 heigth 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

coloration in *P. hispanicus* and other lacertids such *Z. vivipara* (Vroonen *et al.*, 2013). However, we cannot dismiss the possible role of crypsis in hatchlings and striped females because it was not the focus of the present study.

Reticulated-blotched females had larger head height and femoral length than striped females, although any differences disappeared after controlling for body size. These results suggest that differences between morphs are likely to be in general size, although body size differences were only marginally significant (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 with respect to 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 because a trade-off between egg and hatchling size, and between progeny size and number, may exist in lizards (Svensson *et al.*, 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 because 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. Because we did not find sex ratio differences between morphs, the effect of maternal colour 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 to research focusing on dorsal pattern polymorphism in other lizard species (Lepetz *et al.*, 2009; Cox & Calsbeek, 2011). Correspondences between colour 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 of the available evidence shows that dorsal coloration is generally presumed to be an adaptation for crypsis or thermoregulation in diurnal reptiles (Norris, 1965; Kettlewell, 1973; Jackson, Ingram & Campbell, 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 behaviour (Ortega *et al.*, 2014). This finding 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 because 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. Despite melanin-based coloration being 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 considered 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 to 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 ventrolateral 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 suggests 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 a different morphology because 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 the 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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