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TESIS DOCTORAL 2015

Jesús Ortega Giménez

DETERMINANTS OF PHENOTYPIC VARIATION IN THE IBERIAN WALL LIZARD SPECIES COMPLEX (*PODARCIS HISPANICUS*)

Determinantes de la variación fenotípica en el complejo de especies de la lagartija ibérica (*Podarcis hispanicus*)

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Jesús Ortega Giménez

Tesis doctoral

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UNIVERSITAS COMPLVTENSIS MATRITENSIS

FACULTAD DE CIENCIAS BIOLÓGICAS

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Madrid, 2015

Jesús Ortega Giménez

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La biología es la ciencia. La evolución es el concepto que hace a la biología singular.

Jared Diamond

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Madrid, 2015

El doctorando

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General section

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Ha sido largo camino y es momento de pararse por un momento a reflexionar. Recuerdo dos sabios consejos que me dieron en mi infancia: para saber algo tienes que leer, luego leer y luego leer más; si quieres ser bueno en algo hay que practicar, practicar y practicar. No puedo dejar de pensar que la constancia y tener claro un objetivo son condiciones indispensables para prosperar, no solo en una carrera científica, sino en cualquier otra disciplina o ámbito de la vida. No obstante, el mérito no es solo mío pues no habría podido llegar hasta aquí sin la ayuda y apoyo de unas cuantas personas.

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General section

Determinants of phenotypic variation in the Iberian wall lizard species complex (*Podarcis hispanicus*)

Introduction

> ***Variations, however slight and from whatever cause proceeding, if they be in any degree profitable to the individuals of a species, in their infinitely complex relations to other organic beings and to their physical conditions of life, will tend to the preservation of such individuals.***
>
> **Darwin CR. 1859**

The study of variation

The study of variation has long fascinated biologists and attempts to account for and explain this variation were major contributors to the formulation of Darwin's ideas of evolution (Russell & Bauer, 2005). Science, and biology in particular, will be always grateful to Charles Darwin and Alfred Russel Wallace, for the, although quite simple, revolutionary idea of evolution by means of natural selection. However, Darwin's influence on modern thought goes beyond this "re-volution". Darwinism rejects all supernatural phenomena and causations. The theory of evolution by natural selection breaks with a static conception of the world that invokes gods as creators or designers, it explains the adaptedness and diversity of the world in a materialistic way (Mayr, 2000).

Essentialism, also known as typology, was the ideology that dominated the thinking of the western world well into the ninetheenth century (Mayr, 1982), and had its roots in platonic philosophy and its Aristotelian refinement (Panchen, 1992). The variable world of phenomena was viewed as the imperfect manifestation of underlying essences, as expressed in form and behaviour and constituted a great problem for biology (Panchen, 1992). Thus variation was viewed as being accidental and consequently of no interest to scientific inquiry (Russell & Bauer, 2005). Essentialism emphasized discontinuity, invariance and stability, validating the reality of taxa independently of an explanatory theory (Panchen, 1992) and is directly connected to Linnaeus's insistence on the reality, sharp delimitation and constancy of species (Mayr, 1982).

Darwin overcame the constraints of essentialism thinking and considered variation itself as an important source of evidence against biological essentialism. He completely rejected typological thinking and introduced an entirely different concept: population thinking (Mayr, 1969). Variation is inherent to any species, and, hence, all groupings of living organisms are populations that consist of uniquely different individuals. Even humans show variation ranging from morphology, coloration, genetics and physiology (Lewontin, 1972; Serre & Pääbo, 2004; Relethford, 2000; Hanihara, 2008).

Evolutionary ecology is a recent discipline which merges ecology and evolution. In fact, this historical perspective made possible that evolutionary ecology blossomed into a massive discipline that has assimilated and largely replaced other ecological subdisciplines (Pianka, 2011). Evolutionary ecology focuses on the study of variation within individuals, among populations and among species. But not all variation is evolutionary significant, only those traits whose phenotype is transmitted from parents to offspring over multiple generations has the potential to evolve. Thus, heritable variation is a requisite for natural selection to act and produce adaptations. This is why evolutionary ecologists are interested in the study of the causes and effects of variation (Mazer & Damuth, 2001).

Variation in life-histories

Life history theory is a branch of evolutionary ecology that seeks to explain how natural selection and other evolutionary forces shape organisms to optimize their survival and reproduction in the face of ecological challenges posed by the environment (Stearns, 1992; Roff, 1992; Stearns, 2000). The most common studied life history traits are: number, size, and sex of offspring, growth rates, age and size at maturity; size- or age-specific reproductive effort, size- or age-specific rates of survival and lifespan. These life-history traits are considered fitness components and its evolution depends on the interaction between selective factors in the environment and factors intrinsic to the organism that affect survival and reproduction (Roff, 1992).

Trade-offs have played a central role in the development of the life-history theory (Stearns, 1989). Trade-offs are defined as the negative correlation between two life-history traits so that the fitness benefit through increasing one trait is balanced against a fitness cost through decreasing the other (Stearns, 1992). All life history traits should always evolve so as to maximize survival and reproduction and thus fitness (Houle, 2001). However, resources are finite and an organism cannot invest maximally in all traits. Selection cannot maximize life history traits beyond certain limits and will select the combination of traits that maximizes fitness. Without trade-offs and other kinds of constraints, the highest fitness would belong to a "Darwinian demon" that would take over the world (Law, 1979) a hypothetical organism for whom such trade-offs do not exist.

One of the most prominent examples of life-history trade-offs refers to the cost of reproduction. The cost of current reproduction is two folded: costs paid in survival and costs paid in future reproduction (Stearns, 1989). Species that reproduce early in life have a shorter life span (e.g. mice versus humans) and there is a negative correlation between early and late fecundity (e.g. dandylion versus holm oak) (Stearns, 1992).

Variation in life history trait is not only caused by trade-offs and constraints. In some cases, variation may be environmentally induced by extrinsic factors (phenotypic plasticity) (Losos *et al.,* 2000; Via & Lande, 1985), in others they are mediated by intrinsic differences linked to geographically variable conditions (local adaptation) (Travis, 1994); or a combination of both (Sears & Angilletta, 2003). Hence, the understanding of the ecological and evolutionary significance of intraspecific geographic variation in life history traits requires teasing apart genetic and environmental sources of the variation (Berven et al., 1979, Ferguson & Brockman, 1980; Ballinger, 1983; Berven & Gill 1983; Jones & Ballinger, 1987; Ford & Siegel 1989). Given these evidences, life-histories are highly variable not only between species but also between closely related populations (Roff, 1992; Stearns, 1992; Du *et al.,* 2005).

Life history theory has at its core the aim to answer fundamental questions such as: How long should an organism live? How many offspring should it have and how large should they be? How many times should it reproduce? The study of life history evolution is thus about understanding adaptation, the most fundamental issue in evolutionary biology (Fabian & Flatt, 2012).

Antipredatory behaviour

> ***The rabbit runs faster than the fox, because the rabbit is running for his life while the fox is only running for his dinner.***
>
> **Aesop. Aesop´s fables**

Predation is defined as an interaction in which one free-living individual kills and derives resources from another organism (Peter, 2001) and is considered a major selective force in the evolution of animal adaptations (Lima & Dill, 1990; Lima, 1998). Predation is a process, involving several stages, on the part of the predator: detection, identification, approach, subjugation and consumption (Endler, 1991). Predators exert stronger selection pressure on individual prey species than any individual prey species can exert on its predators. The “life-dinner principle” and the asymmetric nature of predator-prey arms races mean that, in general, the prey tend to have the advantage in those particular arms races (Dawkins & Krebs, 1979) (Fig. 1). Among the different traits that can be affected by predation, coloration and behaviour have been studied in depth. The white sands constitute a great example of rapid evolutionary shift in coloration induced by predation. White Sands is a distinctive landscape of stark white hydrous calcium sulfate (gypsum) dunes surrounded by the dark adobe soils of deserts of the Tularosa basin that creates a dramatic ecotone of white and dark substrates (Kocurek, 2007). Here, three lizards species have rapidly evolved light colored convergent phenotypes under strong divergent selection from a brown ancestral phenotype: *Holbrookia maculata* (common lesser earless lizard), *Sceloporus undulatus* (Eastern fence lizard), and *Aspidoscelis inornata* (Little striped whiptail). Presumably, the blanched coloration of lizards at White Sands is an adaptation for crypsis. This is a great example of how sometimes predation avoidance is best facilitated by strategies which result in going undetected by predators matching background coloration. However, predation-mediated selection might also promote the evolution of conspicuous coloration if the fitness benefits of exhibiting bright coloration are greater than the costs (Wilkinson, 2003). Except for aposematic species, as conspicuous coloration makes prey more detectable, conspicuous individuals may compensate for increased predation pressure with antipredation behaviours (Lima & Dill, 1990; Forsman & Appelqvist, 1998). For example, conspicuous colorations may act as a decoy to deflect attacks of predators to non vital areas of the body, increasing the probability of survival (Arnold, 1984; Wilkinson, 2003). This is well documented in some lizards with brightly coloured (red, green or blue) autotomous tails (Pianka & Vitt, 2003). The effectiveness of coloured tails might be enhanced by waving or ondulating tails movements diverting attacks away from the body and, hence, increase escape probability (Arnold, 1984; Cooper & Vitt, 1985; Castilla *et al.,* 1999; Watson *et al.*, 2012). Nevertheless, tail displays may also be a pursuit-deterrence signal that warns predators that they have been detected, prompting predators to abort attacks (Dial, 1986; Hasson *et al.,* 1989; Cooper, 2007).

Because predation risk for any prey increases as the predation sequence proceeds from one stage to the next, and because many prey species are subject to predation by more than one predatory species, selection should be greater for prey defenses that result in early detection of predators. Thus, prey may also evolve antipredator behaviours to avoid detection or successfully avoid predators (Edmunds, 1974; Lima & Dill, 1990; Endler, 1991). To avoid predators, many reptiles escape as soon as the predator is detected, while others allow closer approach of predators before escape (Vitt & Congdon, 1978; Greene, 1988). For example, theoretical models and empirical evidence suggest that prey should not flee immediately upon detecting an approaching predator, but instead should adjust their escape response to minimize the costs of flight because approaching predators do not always pose an immediate threat (Ydenberg & Dill, 1986). However, the most usual response of a lizard when it escapes is to flee away from the predator.

Figure 1. An Uromacer snake captures an Anolis in the Dominican Republic and illustrates the cost-benefit asymetry between predators and prey. Picture credits: Jesús Ortega Giménez.

Escape tactics of lizards can vary within a species according to micro-habitat characteristics (Snell *et al.,* 1988; Bulova, 1994), thermal conditions (Rand, 1964; Hertz *et al.,* 1982; Carrascal *et al.,* 1992), gravidity (Bauwens & Thoen, 1981; Braña, 1993) or ontogeny

(Pounds *et al.,* 1983; Garland, 1985). Escape could be interpreted as successful if the prey has greater speed and stamina than the predator and is able to flee to a refuge where the predator cannot follow it (Edmunds, 1974; Endler, 1991). However, several energetic and perceptual benefits may arise when individuals move in a discontinuous way (Kramer & McLaughlin, 2001). This phenomenon is known as intermittent locomotion and occurs in many other behavioural contexts such as general locomotion, and search or pursuit of prey (Avery *et al.*, 1987). Thus, brief stops during escape could improve predator vigilance and microhabitat perception, and hence, allow prey to adjust their speed or change their sprint direction. In addition, pauses may also cause predators to lose contact with an already detected prey (Edmunds, 1974; Herzog & Burghardt, 1974) via background matching (Martín & López, 1995) or by reducing the ability of predators to anticipate future prey position (Driver & Humphries, 1988).

Polymorphism

> ***If you come to any more conclusions about polymorphism, I shd be very glad to hear the result: it is delightful to have many points fermenting in one's brain.***
>
> **Darwin CR. to Hooker JD.(25 Feb 1846)**

In contrast to continuous traits, discrete polymorphisms are excellent model systems to examine micro evolutionary processes (Iserbyt *et al.,* 2013). They provide tractable systems because they are easy to categorize and, hence, simplify the study of evolutionary dynamics in the wild (Calsbeek *et al.,* 2010). A species is polymorphic when in a population individuals of the same age and same sex display one of several phenotypic variants that are genetically inherited and for which the expression is sensitive neither to the environment nor to body condition (Roulin, 2004). Polymorphisms affect morphological, physiological or behavioural traits (Galeotti *et al.,* 2003) and can be found at the species, population, ontogenetic or sex level (Forsman & Shine, 1995). Among them, colour polymorphism is one of the most studied as it is a widespread phenomenon in many animal taxa.

Color polymorphism is a common phenomenon in vertebrates (Huxley, 1955; Forsman & Shine, 1995; Fuller & Travis, 2004; Roulin, 2004; Hoekstra *et al.,* 2006; Hoffman *et al*., 2006) (Fig. 2). Individuals expressing alternative color patterns are also commonly selected to express alternative behaviours, with different behaviours or reproductive strategies specifically favored in combination with each color pattern (Brodie, 1992; Forsman & Appelqvist, 1998). For example, female plumage coloration covaries with reproductive strategies in the tawny owl (*Strix aluco*) (Roulin *et al.,* 2003) and darker manned lions (*Panthera leo*) are more aggressive and sexually more active (West & Packer, 2002). In lacertids, the existence of several ventral morphs correlated with fitness traits has been extensively studied in some species such as *P. muralis* and *Z. vivipara* (Vercken *et al*., 2007; Lepetz *et al.,* 2009; Galeotti *et al*., 2013), while in others has been completely overlooked (e.g. *P. guadarramae,* Fig. 3).

Sex-specific color polymorphisms represent excellent model systems to study the nature of diversifying selection and have been extensively documented in many different taxa, such as insects, isopods, molluscs and vertebrates (Oliveira *et al*., 2008). Studies on male polymorphisms are abundant, and its maintenance tend to be explained by negative frequency-dependent selection. This means that rare phenotypes experience a mating or survival advantage over more common morphs (Partridge, 1988; Olendorf *et al.,* 2006).

In the Side-Blotched Lizard *Uta stansburiana* aggressive orange males usurp the smaller territories of blue mate-guarders, blue morphs defeat yellow sneakers by guarding mates; and yellow sneakers defeat orange territory holders by cuckolding fertilizations. For example, sneakers do better when territorial males predominate (Sinervo *et al.,* 2001). However recent research has shown that, contrary to this bias towards the study of male polymorphism, polymorphisms restricted to the female sex are more common in nature than previously thought (Kunte, 2009; Svensson *et al.,* 2009). 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). Although the interest in FPP has increased substantially (Svensson *et al.,* 2009; Cox & Calsbeek, 2012; Ortega *et al.,*2014) a bias towards insect studies exists, whereas vertebrates have been less studied (Paemelaere *et al.,* 2011). For instance, female-limited polymorphism is found in several species of damselflies, dragonflies and butterflies. In most of these species, there are two or more morphs, one of which is characterized by male-like coloration and/or patterning (Sirot *et al.,* 2003) and is considered to have evolved as a counter adaptation to reduce costs of harassment imposed by mate-searching males (Iserbyt *et al.,* 2013).

Although most research on lizards has focused on carotenoid-based polymorphisms (e.g. Sinervo & Zamudio, 2001; Vercken *et al*., 2007; Galeotti *et al*., 2013) there are some examples of dorsal (melanin-based) female-limited polymorphism. 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 *et al.,* 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 exists in some lizards, suggesting that distinct selective pressures may exist between male and female dorsal patterns. However, the evolutionary processes underlying female-limited polymorphism are not well understood. It is worth saying that a link between colour polymorphisms has been demonstrated as processes generating and maintaining it can promote speciation. In these examples, speciation is driven by the fixation of one or more morphs (Hugall & Stuart-Fox, 2012). Given all these evidences, the study of polymorphisms in general, and FPP in particular, constitute an exciting and promising area of research. It 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.

Figure 2. These photos show part of the high dorsal pattern variation in the Gargoyle New Caledonian gecko (Rhacodactylus auriculatus). Picture credit: Jesús Ortega Giménez.

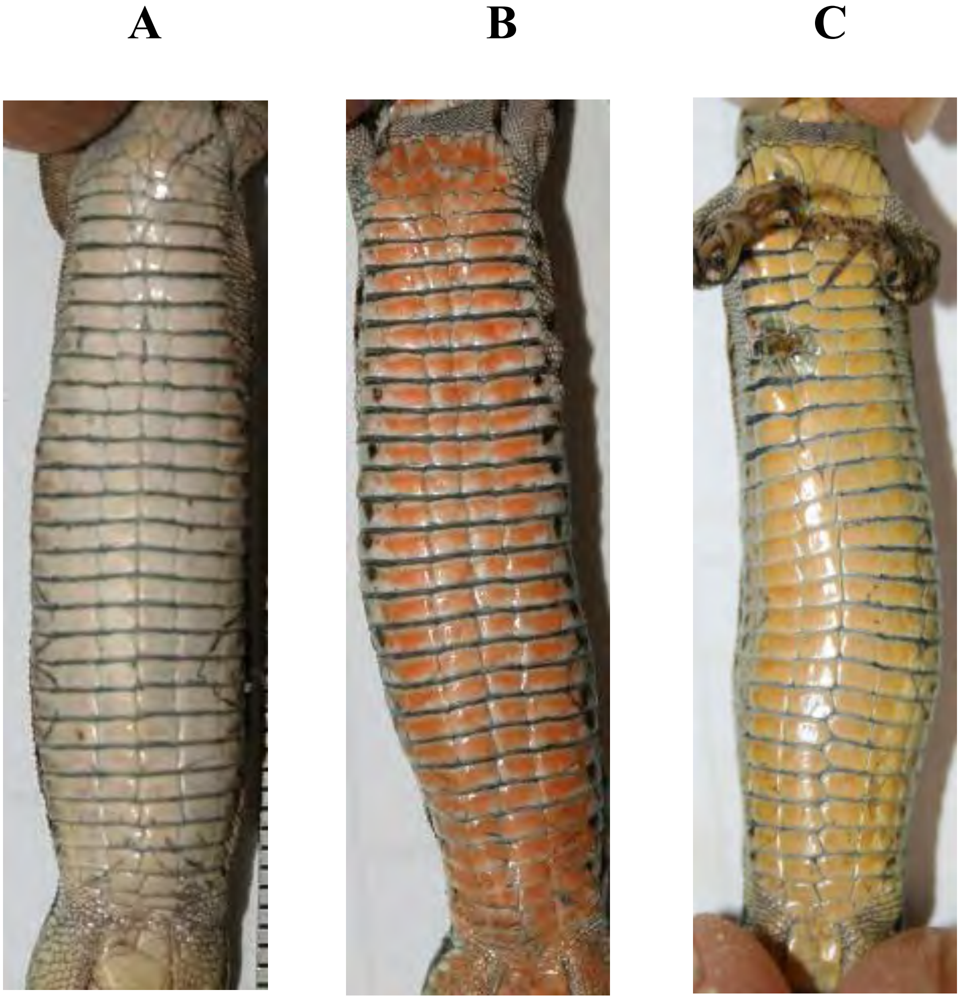

Figure 3. Female restricted ventral polymorphism in P. guadarrame. A) White, B) Red, C) Yellow. Picture credit: Jesús Ortega Giménez.

Sexual signals

Natural selection may explain the evolution of apparently adaptive characters and the process of speciation (Darwin, 1859). However, many characters are apparently disadvantageous and maladaptive, which cannot have evolved by natural selection because they are unlikely to enhance survival (Andersson, 1994; Møller, 1994). Moreover, in most cases individuals having these traits may incur a cost of survivorship, for example for being more conspicuous to predators (Zuk & Kolluru, 1998; Martín & López, 2001). For instance, in the long-tailed widowbird (*Euplectes progne*) females prefer males with longer tails and the long tail impairs male flight (Andersson, 1982). Darwin (1871) hypothesized that these extravagant secondary sexual characters evolved by sexual selection, a process where the disadvantages for survival are balanced by advantages in mating as a result of female preferences (or male preferences in a few cases). Hence, sexual selection deals with the evolution of secondary sexual characters which give certain individuals an advantage over others in relation to reproduction. Specifically, sexual selection comprises two different mechanisms: male-male competition and female mate choice. I am not aware of a better explanation than this Darwin (1871) quote: "*The sexual struggle is of two kinds; in the one it is between individuals of the same sex, generally the males, in order to drive away or kill their rivals, the females remaining passive; whereas in the other, the struggle is likewise between the individuals of the same sex, in order to excite or charm those of the opposite sex, generally the females, which no longer remain passive, but select the more agreeable partners*". The direct consequence of this dialogue both within and between sexes is the evolution of sexual signals that convey information of the signaler. Sexual signals can be present in any sensory channel: vision, touch, audition or smell. Nonetheless, the study of sexual signals is plagued by examples of bright and colourful animal traits such as the nuptial colours of cichlids and birds of paradise. This is certainly due to our bias as human observers. We are attracted by conspicuous colorations as a byproduct of our own evolutionary history as diurnal primates.

Visual signals

This category comprises behavioural displays and conspicuous colors or patterns. Many diurnal territorial lizards, particularly from the agamid and iguanid lineages, perform stereotyped displays that may have several functions, such as species identification, mate attraction or mediate agonistic interactions (Radder *et al.,* 2006). For example, *Anolis* lizards do "head-bobbing" or "push-ups" displays in which the head is moved up and down in a stereotyped temporal pattern (Jenssen, 1977). However, we will focus on coloration *per se,* such as the coloration of the *Anolis* dewlap that maximizes an already existing visual signal (Fig. 4).

In reptiles, four basic types of pigment cells have been identified: xanthophores, erythrophores, iridophores, and melanophores (Cooper & Greenberg, 1992; Bagnara, 1998). Xanthophores and erythrophores are light-absorbing pigment cells that contain pteridines and carotenoids, giving yellow to red coloration (Kuriyama *et al.,* 2006). Iridophores are light reflecting pigment cells, responsible for structural colors that contain light-reflecting platelets made of crystalline purines and pteridines (Bagnara, 1998). Melanophores are light-absorbing pigment cells that absorb the most light to produce black or brown colors. The spatial arrangement and architectural combination of these pigment cells can produce a wide array of skin colors in reptiles (Morrison, 1995; Morrison *et al.,* 1996).

Coloration may function as a sexual signal that mediates intra- and inter- sexual communication as well as population recognition (Rosenblum, 2009). For example, adult male "green lizards" (*Lacerta schreiberi* and *L. viridis*) have green brilliant dorsolateral colorations (Václav *et al*., 2007; Martín & López, 2009) (Figure 4). Characteristics of this coloration, which is often carotenoid-dependent, may reflect the health of a male (Ressel & Schall, 1989; Martín *et al.,* 2008; Martín & López, 2009) and might be potentially used by females to select mates.

Figure 4. Examples of sexual visual signals: conspicuous dorsolateral coloration in Lacerta Schreiberi and dewlap yellow coloration in Anolis capito. Picture credit: Jesús Ortega Giménez and Roberto García-Roa.

Chemical signals

Chemical signals are commonly used by many animals during social and sexual behaviours (Wyatt, 2003; Mason & Parker, 2010). Thus, chemoreception is one of the main sensory systems used by most animals, including many vertebrates, and chemical signals (pheromones or semiochemicals) play an important role in communication and sexual selection (Wyatt, 2003; Müller-Schwarze, 2006; Mason & Parker, 2010). In terrestrial vertebrates, pheromones are very often incorporated into faeces, urine or other scent marks left on the substrate with the purpose of marking territories boundaries or attracting mates (Lopez *et al.,* 1998; Brennan & Kendrick, 2006).

In lizards, pheromones are deposited on the substrate by epidermal structures (pores) located on the ventral surface near the cloaca. Indeed, the ventral location of these pores suggests that secretions are passively deposited on the substrate as lizards move through their home ranges. Depending on exact location we can refer to cloacal, precloacal or femoral pores (Fig. 5). In lacertids, they are located in the ventral part of the thigh and, hence, we call them femoral pores. They have been extensively used in taxonomy as a diagnostic trait, but relatively little is known of their functional significance (Alberts, 1993). Femoral pores are connected to glands that are formed by an invagination of the *stratum germinativum*, which forms a follicular unit, and produce copious amounts of holocrine secretion (Mason, 1992). These structures are widespread in Squamata and also present in phylogenetically distant groups, such as geckos, skinks, agamids, iguanids, lacertids and teiids. The only groups that do not bear them are snakes, monitor lizards and affine taxa (*Heloderma* and *Lanthanotus*). The secretory activity of the femoral glands is greater in the breeding season, males produce more secretion than females, which often lack these structures, and androgens can influence their development and maintain their activity (Cole, 1966; Van Wyk, 1990; Alberts, 1993).

Despite the chemical composition of these secretions is still unknown for most of the species, the rising interest in lizard chemical communication has shown that secretions are composed of both lipids and proteins, although lipids are the main compounds involved in pheromonal communication (Mason, 1992; Martín & López, 2006c). The presence of specific compounds and the relative proportion of each chemical vary widely between species. These variations may be due to phylogenetic differences among species *per se*, but it is also likely that, if the persistence of scent marks in the habitat is a requisite for their efficiency, there is selection for the ocurrence of different chemicals with alternative properties in different habitats (Alberts, 1992; Escobar *et al.,* 2003; Martín & López, 2006b). Indeed, interpopulational differences in chemical signals is quite obvious, even in relatively close populations experiencing contrasting climatic conditions (e.g. Gabirot *et al.,* 2013). Thus, chemical signals involved in scent marking are expected to evolve ensuring that signals are more efficient by being perfectly tuned to, for example, local humidity and temperature (i.e. affecting their volatility and therefore their persistence and transmission through the environment) (Regnier & Goodwin 1977; McDonough *et al.,* 1989; Alberts 1992). Thus, it has been suggested that the observed interspecific or interpopulational differences in chemical signals of lizards might partly reflect selection for maximizing the efficacy of signals in different climatic conditions (Alberts, 1992; Escobar *et al.,* 2001; Escobar *et al.,* 2003; Martín & López, 2006b; Gabirot *et al.,* 2012) as chemical signals must be detectable, transmitting effectively through an environment which tends to distort or attenuate the signal (e.g. Morton, 1975).

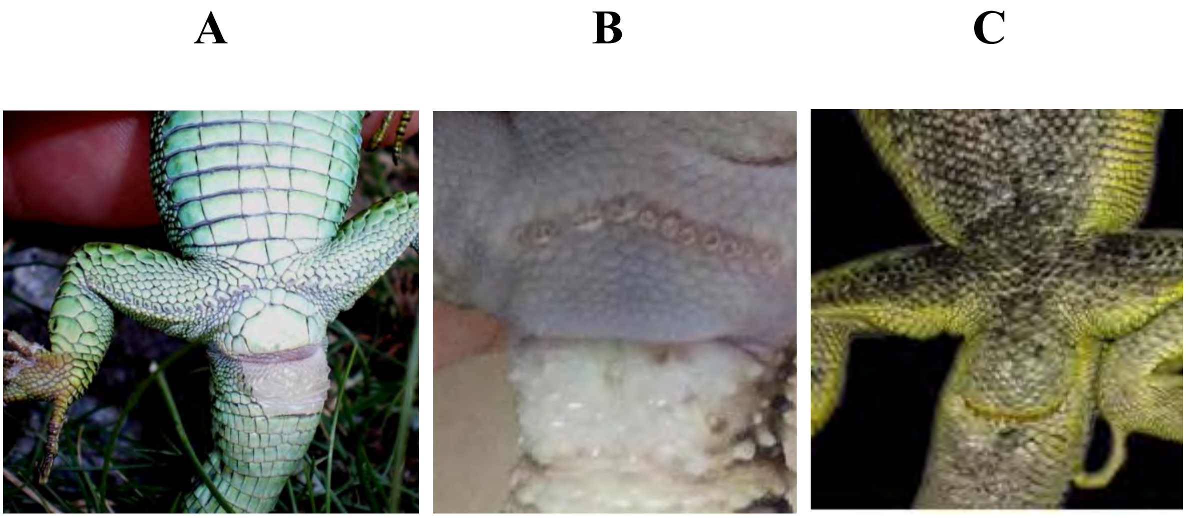

Figure 5. Diversity of epidermal pores. A) Femoral, in Iberolacerta cyreni; B) Precloacal, in Eublepharia macularius; C) Precloacal, in Liolaemus aff. melanops. Picture credits: Jesús Ortega Giménez, Elena fraile and Roberto García-Roa.

Objectives

The main goal of this thesis is to study the factors that shape phenotypic variation in the Iberian wall lizard (*P. hispanicus*) species complex. To accomplish this, the thesis is organized in six chapters with the following concrete objectives:

Chapter I. To compare the reproductive investment and unravel the underlying basis of the altitudinally divergent phenotypes of two proximate *P. guadarramae* populations along an altitudinal gradient with contrasted climatic conditions. We aim to disentangle the relative contribution of local adaptation vs phenotypic plasticity in this system.

Chapter II. To examine the influence of proximate effects of local environmental conditions on hatchling growth in lizards from the two populations commented above. We also measure food availability in both sites to determine the relationship between ecosystem productivity and growth.

Chapter III. To determine the adaptive value of conspicuous colourful tails and dorsal pattern morphs and its relationship with escape behaviour in juvenile lizards *P. guadarramae*. We also aim to examine the influence of source population (highland vs lowland) in tail coloration and escape behaviour.

Chapter IV. To test the hypothesis that selection acts differentially among *P. guadarramae* female morphs to create alternative morph-specific phenotypic optima at different levels, by testing whether morphs differ in several fitness proxies. We also examine morphology and growth of hatchlings born from the two female morphs.

Chapter V. To examine phenotypic differences between highland and lowland populations in *P. liolepis*, another species within this species complex. We focus on morphology, ventral and dorsal coloration. We wonder if the pattern of altitudinal variation found in *P. guadarramae* from central Iberia is the same in this species.

Chapter VI. To examine the efficacy (i.e., persistence) of sexual chemical signals of males (i.e. femoral gland secretions) at different temperatures and humidity levels to test the hypothesis that chemical signals are tuned to the local environmental conditions (highland vs lowland).

Material and methods

The general methods used are briefly described in this section. A more detailed description of the methods used in each experiment will be found in each chapter.

Study species: The Iberian wall lizard

The Iberian wall lizard, *Podarcis hispanicus* (Steindachner, 1870), is an heliothermic, insectivorous and small diurnal lacertid lizard that is variable in both morphology and life history characteristics (Pérez-Mellado, 1998). It is distributed througthout the Iberian Peninsula and North-West Africa (Guillaume, 1987) (Fig. 6). It is a saxicoluos species, which means that thrives on rocky substrate, and it can be also found living in anthropogenic habitats (i.e. rocky walls, city parks and buildings) (Van Damme *et al.,* 1997). Molecular and morphological studies have revealed the complex evolutionary history of *P. hispanicus*, and indicate that it is composed of several genetically distinct lineages, many of which warrant specific rank (Oliverio *et al.,* 2000; Sá-Sousa, 2000; Harris & Sá-Sousa 2001, 2002; Harris *et al.,* 2002a, 2002b; Sá-Sousa *et al.,* 2002; Busack *et al.,* 2005; Pinho 2007; Pinho *et al.,* 2006, 2007b, 2008; Renoult *et al.,* 2009; Kaliontzopoulou *et al.,* 2011). Even morphological analysis reveal high levels of variation both within and between mitochondrial lineages which makes difficult to find diagnostic characters (Kaliontzopoulou *et al.,* 2012).

Iberian populations of the *P. hispanicus* complex currently include seven named species (Geniez *et al.,* 2014). *Podarcis bocagei* is found in north-eastern Spain and north Portugal (Galan, 1986; Sa-Sousa, 1998). *P. carbonelli* inhabits western Iberian Peninsula and shows a highly fragmented distribution. It ranges from the Western Central System in Spain and Portugal (Pérez-Mellado, 1981), through the northern coast of Portugal south of the Douro river southwards along the Portuguese coast (Sá-Sousa, 2000, 2001), and an isolated population in Doñana (Harris *et al.,* 2002). *P. vaucheri* occurs in the Baetic mountains and adjacent areas south of the Rio Guadalquivir, which is conspecific with North-African populations (Oliverio *et al.,* 2000; Busack *et al.,* 2005). *P. hispanicus* (*sensu stricto*) has been restricted to the Spanish Levant form (Geniez *et al.,* 2007) although it comprises two alternative mithochondrial lineages: sensu stricto and Galera. So the link between morphological units and genetic lineages is not clear (Geniez *et al.,* 2007). *P. liolepis* is distributed along the Mediterranean Spanish coast from Valencia to Catalonia, westwards to the Basque Country, the Ebro Valley and the Castilian plateau; and in south-eastern France down the Rhone river (Carretero *et al.,* 2006a; Renoult *et al.,* 2010; Kaliontzopoulou *et al.,* 2011).

The lineages formerly known as *P. hispanicus* type 1 (sister taxa of *P. bocagei*) and *P. hispanicus* type 2 (sister taxa of *P. carbonelli*), have been raised to the species status and named as *P. guadarramae* and *P. virescens*, respectively (Geniez *et al.,* 2014). *P. guadarramae* occurs in central and north-western Spain, while *P. virescens* inhabits south-central Spain south of the Iberian Central Mountains (Pinho *et al.,* 2007b). *P. guadarramae* has been further separated in two subspecies: *P. g. guadarramae*, which is only found in the Central Iberian Mountains (Spain) and *Podarcis g. lusitanicus*, which occurs in northern Portugal and north-western Spain (Pinho *et al.,* 2006, 2007b, 2008).

The evidence for hybridization of the different forms is limited and due to past introgression events (Pinho *et al.,* 2006, 2007a, 2007b, 2008, 2009, 2011; Kaliontzopoulou *et al.,* 2011). It is worth noting that the unnamed forms within *P. hispanicus* are well supported by genetic analyses and could be true species (Salvador & Carretero, 2014). The complicated taxonomy of this group will benefit from the study of contact zones and interdisciplinary research.

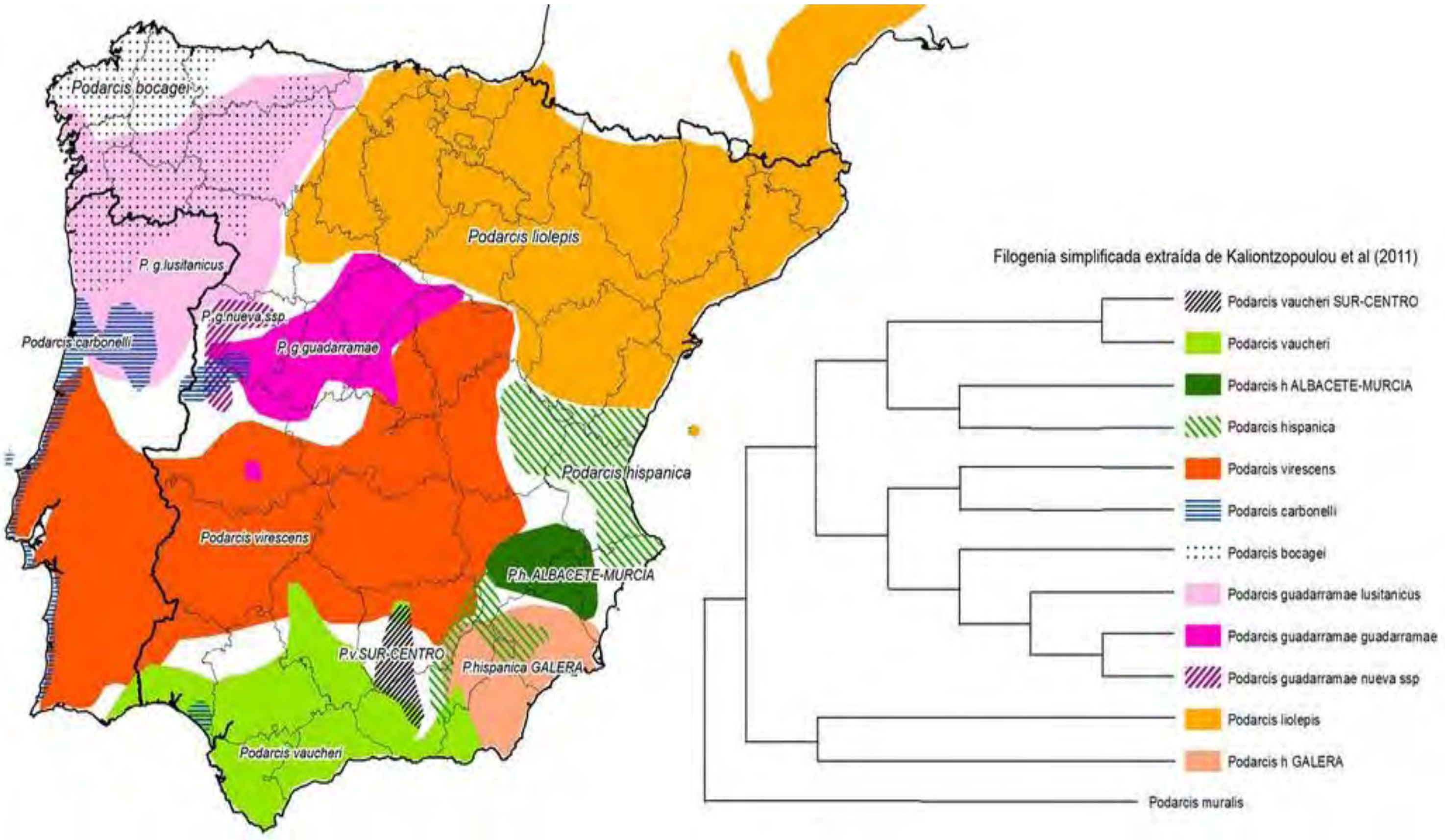

Figure 6. Map showing the distribution of the different lineages and species in the Iberian Peninsula and south-eastern France. Note that the term hispanica is misused here, instead of hispanicus. Map credits: Vicente Sancho, http://esoescomotodo.jimdo.com/reptiles/podarcis-hispanica/.

In the present work we focused on *P. g. guadarramae* and *P. liolepis*. Overall, the *P. hispanicus* species complex, with its wide geographical distribution and the high morphological and genetic diversity, both within and between lineages, offers a great opportunity to shed light on the evolutionary processes underlying phenotypic variation.

Study sites

Guadarrama Mountains

The 'Sierra de Guadarrama' is a mountain range forming the main eastern section of the Sistema Central, the system of mountain ranging at the center of the Iberian Peninsula. It constitutes a natural division between the North and South mesetas of the Iberian Peninsula, and is located between the Sierra de Gredos in the province of Ávila, and Sierra de Ayllón in the province of Guadalajara. The range runs southwest-northeast, extending into the province of Madrid to the south, and towards the provinces of Ávila and Segovia to the north. The chain as a whole measures approximately 80 km in length, with its highest peak, Peñalara, reaching 2,428 m above sea level.

Two nearby populations of *P. guadarramae* in the Guadarrama Mountains (Central Spain) focused the vast majority of the research presented in this memory (Fig. 7) . These sites are separated 6 km by air at different altitudinal ranges. The 'lowland' locality is located in an oak forest ('La Dehesa de la Golondrina´) near Cercedilla village (40º44´N, 04º02´W; 1,250 m altitude), where lizards can be found on granite rocky outcrops inside the forest. This locality has a mean annual temperature of 10.9 ºC and a mean annual rainfall of 674 mm (data from 'Embalse de Navacerrada' meteorological station, less than 1 km from the study site). The 'highland' locality is found in the upper part of a mountain valley (`Fuenfría') (40º47´N, 04º03´W; 1,750 m altitude) occupying granite rock walls and rock piles at the edge of a pine forest. This area includes the `Mirador de la Reina', and is enclosed in the recently protected area of the `Guadarrama National Park'. In this population, mean annual temperature is 6.2 °C, and mean annual precipitation is 1170 mm (data from 'Puerto de Navacerrada' meteorological station, 3 km from the study site).

Ariège

The Ariège River gives its name to this area. Ariège is located in southwestern France and limits in the south with Spain an Andorre. It is one of the 8 departments that are included in the Midi-Pyrénées region, the largest region of Metropolitan France by area. A vast area within Ariège was declared regional natural park in 2009. Three main areas can be distinguished: the plains, the Pyrenean foothills and the high Pyrenees.

Figure 7. Sampling localities in central Spain and south-western France. Picture credits: Google Earth.

In search of brown lizards (*P. liolepis*), we sampled three different localities in Ariège: `Pêch de Foix´, `Le Mas d´Azil´ and `Col de Chioula´ (Fig. 7). `Pêch de Foix´ (42°57'46.79"N, 1°37'22.17"E; 800 m a.s.l.) and `Le Mas d´Azil´ (43° 4'22.17"N, 1°21'29.47"E; 400 m a.s.l.) are lowland sites in an area mediterranean climatic influence which are located at the foothills of the Pirynees. The predominant vegetation are oak trees (*Quercus* sp) and box (*Buxus sempervirens*). `Col de Chioula´ (42°44'53.20"N, 1°50'25.58"E; 1700 m a.s.l.) is a highland site in the Pyrenees where vegetation is dominated by beech forests (*Fagus sylvatica*) with scattered European silver firs (*Abies alba*).

In all these populations brown lizards can be found on rock cliffs and boulders, and sometimes on the ground, but never associated to human constructions.

Lizard capture

Once a lizard was detected, it was approached carefully and captured with a noose which was made of a piece of thread tied to the tip of a fishing rod. The noose was slowly placed around the lizard's neck and then the fishing rod was gently lifted so the noose reduced its diameter and trapped the lizard. After that, the desired measurements and samples were taken or the animal was placed in a tissue bag for latter analyses and brougth to the El Ventorrillo field station or to the Station d'Ecologie Expérimentale du CNRS à Moulis, for *P. guadarrame* and *P. liolepis*, respectively.

Adult lizard husbandry

Immediately after capture, individuals were transported to "El Ventorrillo" field station facilities about 5 km away from the capture localities. Lizards were kept separately in climatic chambers (Ibercex V-450-D walk-in chambers; ASL S.A., Madrid, Spain) (Fig. 7) where temperature and photoperiod were easily controlled automatically. Lizards were individually housed in plastic terraria (40x30x25 cm) filled with a moistened coconut fiber substrate and provided with a water bowl and a brick (24 x 11 x 8 cm) that allowed shelter and climbing opportunities. A 50 W halogen lamp was suspended over one end of the terrarium providing a diurnal temperature gradient (21-45 ºC) allowing thermoregulation of lizards within the preferred temperature range of this species (34.4 °C; Bauwens *et al.,* 1995). In addition, a fluorescent bulb in each shelve provided ambient lighting mimicking the natural photoperiod, and mercury vapor bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14:00 to 15:30). Adult lizards were daily watered, and fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio molitor*) *ad libitum*, dusted with a commercial vitamin and calcium supplement.

Hatchling lizard husbandry

Hatchlings were kept in similar conditions to adults but fed with insects of appropriate size. If the climatic chambers mentioned above were not available, an empty room was arranged and exclusively dedicated to the lizards.

Outdoor enclosures

In the reciprocal transplant experiment, four outdoor enclosures were built to raise hatchlings under natural conditions. Two were located in the lowland and the other two in the highland site, in the typical habitat were lizards are found. Galvanized metal walls of 85 cm, partly buried in the floor (25 cm) delimited its 25 m^2 of surface (Fig. 8). A net covered the enclosures from above to avoid bird predation. Five artificial boulders per enclosure, each one composed of four concrete bricks (50 x 25 x 25 cm) and covered with granite rocks, were added to mimic the saxicolous habitat of the species. Under each boulder we excavated a pit of 25 cm to fit a clay brick (40x30x25 cm) with 6 longitudinal holes to provide additional underground shelter.

Figure 7. Lizard facilities. Two sets of shelves as the one shown in this photo were arranged in each climatic chamber.

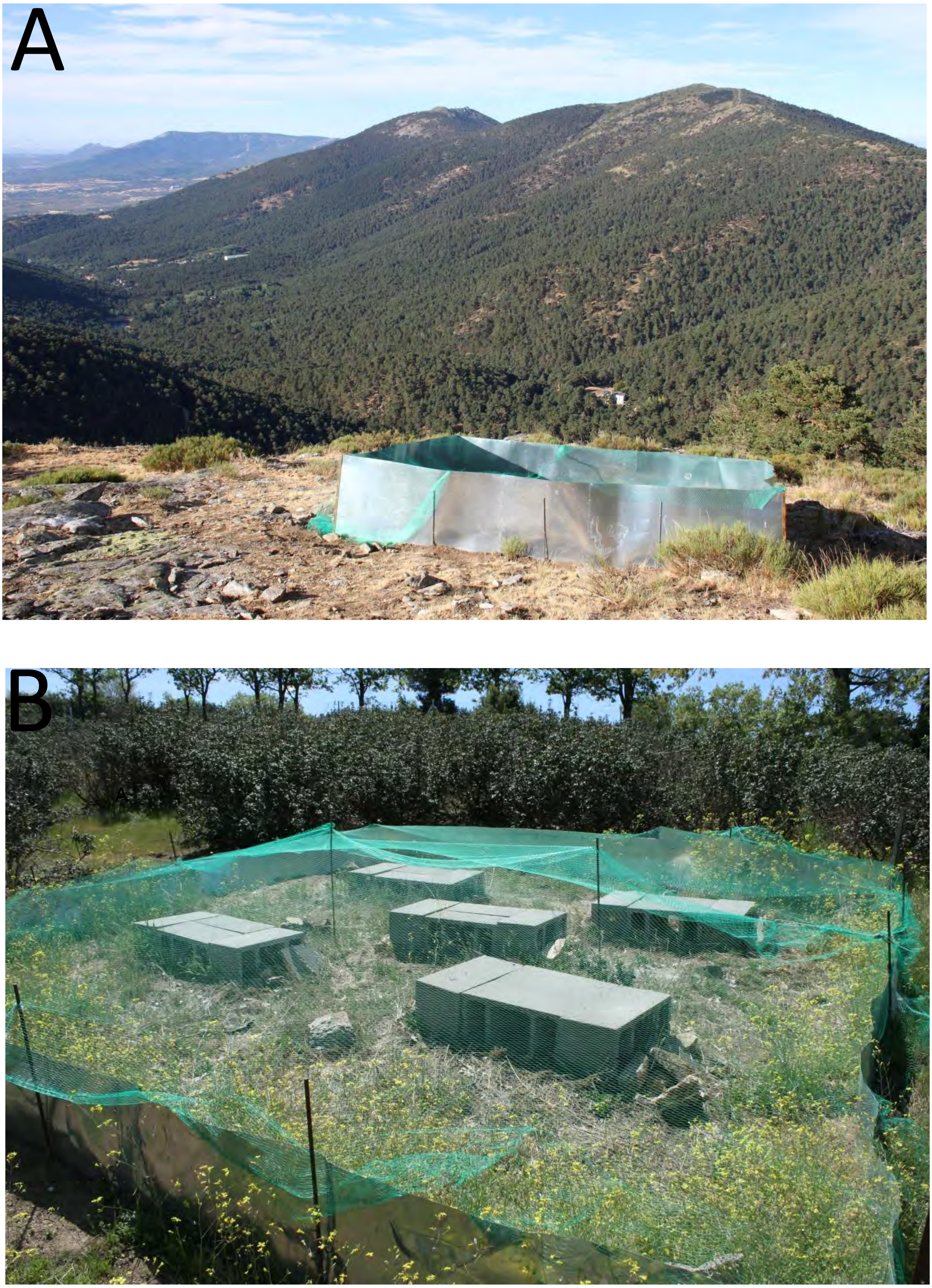

Figure 8. Outdoor lizard enclosures. A) Puerto de La Fuenfría (highland site); B) Dehesa de La Golondrina (lowland site).

Escape behaviour

We simulated a predator attacking from behind by tapping lizards close to the tail with a brush to stimulate them to run along a 1 m treadmill with a cork substrate that provided excellent traction. Cenital filming was performed with a miniDV video camera (JVC GR-DVL365EG, Japan) recording at constant 25 frames s^{-1}. Before the trials, we standardised body temperature of lizards by placing hatchlings in an incubator at 35.5 °C for 30 min, which is the optimal temperature for sprint performance in *P. hispanicus* (Bauwens *et al.,* 1995). Each lizard was run four consecutive times with at least a 10 min resting period between successive trials to avoid stress and fatigue (Braña & Ji, 2000). Videotapes were examined frame by frame, and we noted distance, time moved and tail waving behaviour by lizards using the tip of the snout as a reference (Martín & Avery, 1998).

Immune response

To assess the immune response of lizards we used the the phytohaemagglutinin (PHA) injection test (Svensson *et al.,* 2001; Belliure *et al.,* 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 post-injection measures (Smits *et al.,* 1999; Belliure *et al.,* 2004).

Chemical analyses

We extracted the femoral secretions by gently pressing with forceps around the femoral pores, and collected them directly in glass inserts that were placed into glass vials with Teflon-lined stoppers. Vials were stored at -20ºC until analyses could be carried out. We also used the same procedure on each sampling occasion but without collecting secretion, to obtain blank control vials that were treated in the same manner to compare with the lizard samples in order to exclude contaminants from the handling procedure or from the environment, and for examining potential impurities in the solvent or laboratory equipment. Before the analyses, we added 250 µl of n-hexane (Sigma, capillary GC grade) to each vial. We analyzed lipophilic compounds in samples using a Finnigan-ThermoQuest Trace 2000 gas chromatograph (GC) fitted with a poly (5% diphenyl/95% dimethylsilox- ane) column (Thermo Fisher, Trace TR-5, 30 m length X 0.25 mm ID, 0.25 mm film thickness) and a Finnigan-ThermoQuest Trace mass spectrometer (MS) as a detector. Sample injections (2 µl of each sample dissolved in n-hexane) were performed in splitless mode using helium as the carrier gas at 30 cm/sec, with an injector temperature of 300ºC. The oven temperature program was as follows: 50ºC isothermal for 3 min, then increased to 300ºC at a rate of 5ºC/min, and finally isothermal (300ºC) for 15 min. Ionization by electron impact (70 eV) was carried out at 280ºC. Mass spectral fragments below m/z = 39 were not recorded. Initial identification of secretion components was done by comparison of mass spectra in the NIST/EPA/NIH 2002 computerized mass spectral library. Identifications were confirmed by comparison of spectra and retention times with those of standards from Sigma-Aldrich Chemical Co. Impurities identified in the solvent and/or the control vial samples are not reported. For the statistical analyses of femoral secretions, the relative amount of each component was determined as the percent of the total ion current (TIC).

Coloration measurements

We used an Ocean Optics USB 2000 spectrophotometer to measure reflectance using a dual DT-1000-MINI Deuterium–Halogen light source (Ocean Optics, Inc., Dunedin, FL, USA). A custom-made probe holder oriented at 45º and 1 cm away from the skin surface was used to exclude ambient light and standardise measuring distance. Each spectral reading consisted of percent reflectance recordings in reference to a white standard.

Spectral raw data were processed with CLR 1.1. software (Montgomerie, 2009) and reflectance readings from 300 to 700 nm, summarized over 5 nm steps ("binned"; Grill & Rush, 2000), were selected for analysis, as they represent the spectral range visible to squamates and their avian predators (Bennett & Cuthill, 1994; Cuthill *et al.,* 1999).

Melanin-based coloration

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. 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 lighter pheomelanins bands, which corresponded to striped or blotched surface. The three different types of melanin were measured (in pixels) using the 'magic wand' option (tolerance 32), in order to obtain an accurate estimate of the percentage of each class of melanin by respect to the total of the selected surface.

Results and discussion

Altitudinal variation in adult phenotypes and life-histories

High altitude adult lizards of both sexes were larger with a robust head morphology and had longer femora. **Chapter I** provided clear evidence that the divergent adult body sizes are not driven by size at hatching, which is not contributed to by egg size, nor intrinsic post-hatching growth rates associated with the environmental conditions experienced in our common garden experiment. Female *P. guadarramae* from our highland population laid larger, heavier and more voluminous clutches, and these differences were shown to be independent of the larger size of highland females. As with body size, there is growing evidence that this pattern of variation in reproductive traits (i.e. larger lizards laying larger clutches) may be caused by environmental differences in productivity. Tropidurid lizards show a positive relationship of clutch and female body size with precipitation, possibly via effects on primary production (Brandt & Navas, 2011). In the lacertid lizard *Psammodromus algirus*, female body size and clutch size were larger and positively associated with a gradient of increasing humidity and decreasing temperature (Díaz *et al.,* 2011). Similarly, *P. guadarramae* reproductive investment phenotypes may be influenced by environmental differences in productivity.

In **Chapter I** I also do not find differences in growth between high and low altitude hatchling lizards under standardized laboratory condition. Thus results suggest that adult phenotypes are not the result of intrinsic differences but the consequence of a plastic response. Many other studies agree with our finding and show that growth differences among populations and adult body size are likely a plastic response and not a product of intrinsic differences (Niewiarowski & Roosenburg, 1993; Sorci *et al.,* 1997; Lorenzon *et al.,* 2001).

Chapter II shows that *P. guadarramae* hatchlings grew faster in the higher altitude environment, irrespective of their population of origin. This results confirm that environmental conditions and their effects on primary production may play a key role in maintaining phenotypic divergence in *P. guadarramae*. Specifically, I found that resource limitation at the lowland site seemed to be responsible for differential growth rates and divergent body size between populations. Food availability was higher at the highland site and fluctuated seasonally. Prey availability was correlated with lizard growth. Size-specific, mass-specific growth rates and prey availability were maximal in late spring. Morevover, prey diversity was also higher in spring than in late summer, so I concluded that highlands bear also a better quality of food, considering that a higher diversity of arthropods is beneficial for a generalist species like *P. guadarramae* (Salvador & Carretero, 2014). Several studies support the occurrence of a link between growth rates and habitat production via climate effects that may be responsible for differential adult body size and life histories (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin, 1985; Buckley *et al.,* 2007; Díaz *et al.*, 2011). Specifically, Iraeta *et al.,* (2006) showed that food availability was higher at high altitude compared to lowlands in the same geographical area of my study. Environmental restrictions like drought are common in Mediterranean lowlands and generally decrease with altitude as a result of milder temperatures and higher precipitation, which increases productivity (Nahal, 1981; Iraeta *et al.,* 2012). Resource acquisition constraints occurring at the lowland site, which are more severe in summer, may be responsible for slower growth rates in *P. guadarramae* as it has been demonstrated already in other insectivorous lizard species (Dunham, 1978; Ballinger & Congdon, 1980). However, we cannot dismiss the possible role of limitations on resource harvest rates at the lowland site because we did not examine whether activity patterns vary seasonaly or between sites. Thus, resource and the harvest being not mutually exclusive, might shape a landscape where lowland sites are characterized by poor environmental conditions for food acquisition and, hence, cause slower growth rates.

Adaptive value of conspicuous colourful tails and dorsal pattern morphs

Chapter III shows that hatchling antipredatory behaviour is influenced by the interactions between dorsal patterns, size and tail conspicuousness. We found that neither sex nor dorsal morphs of hatchling *P. guadarramae* differed in the visible spectra composition of tails. However, hatchling reticulated females have more ultraviolet (UV) reflectance in their tails than striped females and reticulated-blotched males, while striped females have intermediate UV reflectance and males the lowest UV reflectance. Considering that these conspicuous tails very likely are useful to divert predator attacks towards the autotomizable tail (Watson *et al.*, 2012), differences in tail conspicuousness in the UV range may be associated with differential predation risk between sex/dorsal morphs and/or the use of different antipredatory strategies. Several alternative explanations that invoke predation as the major selective force could account for UV reflectance differences, such as differential microhabitat selection or foraging behaviour. Indeed, active foraging behaviour and selection of open microhabitat have been linked to more conspicuous tails in *Acanthodactylus beershebensis* lizards, blue tailed hatchlings foraging actively compared to adults and spend more time in open habitats than dull tailed ones (Hawlena *et al.*, 2006). Also, physiological differences between sexes or trade-offs between other behavioural traits could constraint the evolution of high UV reflectant tails in males.

The differences in UV reflectance between reticulated-blotched hatchlings, both males and females, is in sharp contrast with their escape behaviour similarities as they showed higher mean and maximal speed, and spent less time in pauses than striped females. This suggests an association between dorsal morph and some aspects of escape behaviour independently of the sex. Similarly, in two syntopic forms differing in dorsal pattern of the Moroccan rock lizard (*Scelarcis perspicillata*), spotted individuals have longer flight initiation distances and take more time to abandon shelter because they are shyer than striped lizards (Carretero *et al.*, 2006b). Although we have not assessed endurance, we might interpret the escape behaviour of striped female *P. guadarramae* as based on endurance rather than on sprint speed, which, in contrast, would be the preferred tactic of reticulated-blotched hatchlings. Striped females spent more time paused, which could enhance recovery and strengthen endurance. On the other hand, the lower maximal and mean speed in striped females could allow them to escape for longer times. Thus, endurance while fleeing from a predator could be of great importance in spite of their lower speed. In contrast, hatchling males, together with reticulated-blotched females, had faster mean and maximal sprint speeds and made shorter sprints. Thus we can infer that their escape strategy would not depend on endurance but rather on short flight bursts at the fastest as possible high speed.

In **Chapter III** we also found that the only tail coloration trait affecting escape behaviour was tail brightness, independently of sex or dorsal morph. Most studies show that visual conspicuousness of lizards correlates positively with shyness, particularly in males (Hedrick, 2000; Cuadrado *et al.*, 2001; Lindström *et al.*, 2007; López *et al.*, 2005). Cabido *et* *al.* (2009) found that male rock lizards *Iberolacerta monticola* with more shoulder ocelli, which reflect UV radiation, behaved shyer, very likely to compensate for their increased conspicuousness. Our results agree with this hypothesis, hatchlings with brighter tails doing less pauses between sprint bursts than those with duller tails, adopting a shyer behaviour that may minimise predation risk during flight episodes. On the other hand, hatchlings with duller tails may benefit from pauses during locomotion. Several energetic and perceptual benefits may arise when individuals move in a discontinuous way (Kramer & McLaughlin, 2001). Among them, stimulus detection and predator avoidance might explain why hatchling *P. guadarramae* alternate short periods of locomotor activity with short pauses. Pauses may also cause predators to lose contact with an already detected prey (Edmunds, 1974; Herzog & Burghardt, 1974) via background matching (Martín & López, 1995, 1996) or by reducing the ability of predators to anticipate future prey position (Driver & Humphries, 1988). Thus, we can consider two alternative antipredator strategies with relationship to the tail characteristics: hatchlings with relatively duller tails that rely on pauses to confuse predators, and hatchlings with brighter tails that show behavioural compensation, reducing the number of pauses due to their higher detectability.

We did not detect any inter-population differences in tail brightness, UV or bluish coloration of hatchling lizards in spite that contrasting selective forces between populations have been proposed as the cause of geographic variation in the occurrence of bright tail coloration (Brown & Thorpe, 1991). However, lowland males were more prone to tail wave than highland ones. Reduced predation in high elevation localities has been acknowledged in some studies (Van Damme *et al.*, 1989; Fox *et al.*, 1994) and a positive association between prey response and the intensity of threat that they perceive has been widely documented (Stankowich & Blumstein, 2005). Hence, lowland males might suffer a higher predation pressure than their highland counterparts. In addition our results support the prediction that tail waving occurs as a ‘last resort’ strategy that may be related to fatigue in *P. guadarramae* as it is only displayed in the last section of the racetrack by smaller lizards, and sprint speed has no effect in tail waving behaviour.

Chapter **IV** shows that female dorsal morphs in *P. guadarramae* differed in terms of adult morphology, melanin-based coloration and reproductive investment. Striped females, independently of their age, had a higher percentage of melanin (black) and a lower percentage of brownish dorsal coloration than reticulated-blotched ones, while they did 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 addition we detected an ontogenetic trajectory in dorsal melanin-based coloration. Hatchlings had a higher percentage of melanin, and a lower percentage of brownish coloration than adults. As darker reptiles have several thermoregulation advantages (Majerus, 1998; Bittner *et al*., 2002), the thermoregulation constrains 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. guadarramae* 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.

In terms of morphology, reticulated-blotched females had larger head height and femoral length than striped females but these differences disappeared after controlling for body size. These results pointed out that differences between morphs are likely to be in general size, although body size differences were only marginally significant. Reticulated-blotched females, at least regarding these variables, were 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 *P. guadaramae* dorsal morphs had contrasted reproductive strategies. Hatchlings born from reticulated-blotched females were larger and heavier than those born from striped ones. 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). A contrasting physiology (e.g. endocrine profile) between dorsal morphs during egg production might be responsible for this pattern. Overall, research on dorsal pattern polymorphism in other lizard species has never found reproductive investment differences between female morphs (Lepetz *et al*., 2009; Cox & Calsbeek, 2011).

Initially we hypothesized the existence of trade-offs between antipredatory behaviour and reproduction because the cost of gravidity may differ between morphs. However, our results do not support this hypothesis as we did not find egg or clutch size differences between female morphs.

Some studies suggest that fast growth comes at the expense of locomotor performance (Álvarez & Metcalfe, 2007; Shaun *et al*., 2014). However, 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 line with these results, the proportion of dorsal melanin did not influence lizard growth, despite that melanin-based coloration is known to affect the growth of some vertebrates (Ducrest *et al*., 2008).

Chapter IV also suggests that this female-limited polymorphism is not maintained by selective pressures related to immune response. 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) in which males, but not females of *Z. vivipara* with more melanic ventral coloration mounted a stronger PHA-induced immune response.

Altitudinal phenotypes in P. liolepis

Chapter V shows that highland *P. liolepis* were larger and stockier than lowland ones. This result agree with our previous work on the *P. hispanicus* species complex in which we showed that *P. guadarramae* populations followed Bergmann´s rule (Ortega *et al.,* 2014). Thus, deviations from the reversed Bergmann´s clines, as opposed to the vast mayority of squamates (Ashton & Feldman, 2003), might be the general trend in this species complex. In addition, highland *P. liolepis* had more femoral pores than those from the lowlands, a pattern of variation also present in *P. guadarramae* (Gabirot *et al.,* 2012). It is assumed that femoral pore number reflects an investment in and the use of chemical communication (Escobar *et al.,* 2001; Pincheira-Donoso *et al.,* 2008). Hence, selection might adjust femoral pore number depending on the influence of the physical conditions of the substrate and climatic conditions on signal detectability and persistence (Alberts, 1992; Escobar *et al.,* 2003; Pincheira-Donoso *et al.,* 2008). Nonetheless, a recent study found little support for the hypothesis that climate conditions covary with the diversification of femoral pore number across lacertid lizards (Baeckens *et al.,* 2014).

In terms of ventral brightness, lowland lizards were brighter than highland ones in spring whereas the reversed relationship was found in summer. In addition, throat bluish reflectance was higher in summer than in spring. Ventral coloration may function as a sexual ornament or as a status signal (Olsson, 1994; Stuart-Fox *et al.,* 2006) which may explain why bluish radiation reflectance was higher in males for all body regions except for the throat which was similar between sexes. Habitat openness or vegetation cover affect light conditions, which may exert strong selection on visual signals such as ventral coloration to maximize conspicuousness (Marchetti, 1993; Endler & Thery, 1996; Zahavi & Zahavi, 1997; Andersson, 2000) and lead to an increase in the signal-to-noise ratio, or intensity, of communication by changing the structural properties of signals (Ord *et al.,* 2010). Thus, prevailing light conditions may render some ventral coloration variations more effective than others in highland vs lowland habitats, but also between spring and summer, as seasonal variation in body coloration may occur in animals with a distinct reproductive season (Carretero, 2002; Germano & Williams, 2007). We also determined the existence of a ventral gradient of red coloration, with the highest values in the throat and the lowest ones in belly and precloacal. This result is in line with the interpretation that the ventral position of these areas mean that they remain hidden except when the lizard raises its forebody in the presence of a conspecific (Pérez-Mellado & Galindo-Villardón, 1986; Galán, 1995).

With respect to dorsal coloration, lowland lizards were brighter than highland ones in spring whereas the reversed trend was found in summer. This is in accordance with a thermoregulation hypothesis in which the lower temperatures of high altitude sites would favour darker dorsal coloration, as darker reptiles have several thermoregulation advantages such as fast heating rates and higher thermal inertia (Majerus, 1998; Bittner *et al*., 2002). Hence, lowland lizards might have a higher dorsal reflectance at the beginning of the breeding season (spring) as the thermal conditions of their habitat are less restrictive than in the highlands. The same reasoning is argued by Gabirot *et al.* (2013b) for *P. guadarramae* where they found that highland lizards were darker than lowland ones. Lowland lizards also had more greenish dorsal coloration than highland ones and summer lizards. In a similar fashion, summer lizards, with independence of its population of origin, had more brownish coloration. Most evidence shows that dorsal coloration is generally presumed to be an adaptation for crypsis or thermoregulation in diurnal reptiles (Norris, 1965; Kettlewell, 1973; Rosenblum, 2005) and background matching has been widely documented in reptiles (King, 1992; Macedonia *et al.,* 2002; Bittner, 2003). Seasonal variation in body coloration may also occur in species living in habitats where background colours (Wente & Phillips, 2005) or the thermal environment (Fields & McNeil, 1988) change with the time of the year. Thus, background matching might be responsible for these changes in dorsal coloration, due to microhabitat differences (e.g. vegetation) that determine predation mediated selection, or differential habitat selection between populations and seasons. Thus, habitat type, through its influence both on properties of the signalling environment and predation pressure, might be responsible for altitudinal coloration differences in *P. liolepis.*

Efficacy of chemical signals

Chapter VI confirms that differences in chemical composition of femoral secretions between these two *P. guadarramae* lizard populations are due to lower elevation males having higher proportions of cholesterol and fatty acids, and lower proportions of alcohols than higher elevation males. These different compositions should result in different physical-chemical properties of the entire femoral secretion under different temperature and humidity conditions. Thus, under the naturally higher temperatures and drier conditions of the lower elevation population, the higher relative proportion of cholesterol (the main compound in secretions) may contribute to avoid degradation of other more easily alterable compounds that are known to be important in intraspecific communication in this lizard such as cholesta-5,7-dien-3-ol

(López & Martín, 2005; Gabirot *et al.,* 2012a, Martín & López, 2006a). Similarly, the greater abundance of alcohols in secretions of the higher elevation population would not be useful under higher temperatures where alcohols will evaporate more quickly, which may explain why lower elevation males allocated lower proportions of alcohols to secretions. A previous study showed that male *P. guadarramae* from the lower elevation population showed lower chemosensory responses to alcohols than males from the higher elevation population, suggesting interpopulational differences in relative importance of alcohols in communication (Gabirot *et al.,* 2012c). These differences might be linked to the different efficacy of alcohols in scent marks under different climatic conditions.

The fact that these populations that are genetically closely related (Gabirot *et al.,* 2013a) suggests that local adaptation to different environments, and not genetic drift, may drive differences in chemical signals. However, we cannot dismiss the possible role of developmental plasticity as a direct consequence of the environmental thermal conditions experienced by lizards in each population. An experimental study in *Podarcis muralis* showed that differences in basking conditions in the laboratory cause plastic changes in the composition of femoral secretions of males (Heathcote *et al.,* 2014). This suggests that total time spent at optimal body temperatures may affect average metabolic rates and general health of lizards, which would result in a modification of their condition-dependent chemical signals (Martín & López, 2015).

Chemosensory experiments in **Chapter VI** indicate that females detected later (i.e. they had longer latency times) and had lower chemosensory tongue-flick responses to the femoral secretions of males as the time since deposition increased. This showed that the chemical stimuli in secretions faded with time, very likely because chemical compounds that elicit responses evaporated and degraded with time since they were secreted (Epplet *et al.,* 1980). In addition, the loss of detectability and efficacy of the chemical signal was faster under warm temperature and under drier conditions. This is because high temperatures increase evaporation and diffusion rates of chemicals, affecting their persistence (Regnier & Goodwin, 1977; McDonough *et al.,* 1989). Similar detrimental effects of higher temperatures on the efficacy of scent-marks were found in another lacertid lizard species (Martín & López, 2013). In line with these results, high temperatures limit trail-following behaviour of ants by accelerating pheromone decays (van Oudenhove *et al.,* 2011; van Oudenhove *et al.,* 2012).

It is worth noting that these detrimental effects of higher temperatures and drier conditions differentially affected secretion composition in the two studied populations. Thus, secretions from the population at lower elevation, which inhabits warmer and drier environments, seemed to be less affected by these simulated conditions that resembled local ones. In contrast, secretions from the population at higher elevation, which experiences cooler and more humid environmental conditions, suffered a quicker degradation of signal efficiency under climatic conditions similar to those of the lower elevation population. These results support the conclusion that characteristics of femoral secretions are adapted to local temperature and humidity conditions in order to improve their efficiency. Interpopulational differences in the properties of compounds found in the chemical profiles also support this conclusion.

Conclusions

- A common garden experiment shows that divergent body sizes of *P. guadarramae* adult lizards from two populations located at different altitude are not driven by size at hatching, which is not contributed to by egg size, nor intrinsic post-hatching growth rates associated with the environmental conditions experienced in the experiment. Thus, interpopualtional differences in morphology of adult phenotypes are not the result of intrinsic differences but the consequence of a plastic response.

- A reciprocal transplant experiment shows that the growing environment determines growth rates, independently of the population of origin, in two closely related populations of *P. guadarramae* along a 500-m altitudinal gradient with contrasting environmental conditions. The drivers of growth rate differences are likely to be between-sites differences in abundance and diversity of arthropods (i.e. food availability and quality).

- Overall we provide evidence for the existence of sexual and intermorph dimorphism in tail ultraviolet colour reflectance of hatchling *P. guadarramae* lizards. Tails of hatchling reticulated-blotched females have more UV reflectance in their tails than striped females and reticulated-blotched males. Striped females´ tail has intermediate UV reflectance and males the lowest UV reflectance. In addition, we identify sex/dorsal morph, body size and brightness as predictors of different aspects of escape behaviour, and two alternative escape strategies between striped and reticulated-blotched hatchlings that are probably dependent on dorsal morph differences, independently of sex. Reticulated-blotched females and males, run faster and spend less time paused than striped females, two aspects that might reflect an escape behaviour strategy based on endurance in striped females. Larger hatchlings run longer distances and tail wave less than smaller hatchlings. In addition, lowland males display tail waving as a ‘last resort’ antipredatory strategy that may be related to fatigue. Moreover, hatchlings with brighter tails take fewer pauses between sprint bursts than those with duller tails, adopting a shyer behaviour that may minimize predation risk during flight episodes. Hence, hatchling antipredatory behaviour is influenced by the interactions between dorsal patterns, size and tail conspicuousness.

- The two melanin-based dorsal morphs of female *P. guadarramae* have contrasted reproductive strategies. Hatchlings born from reticulated-blotched females are larger and heavier than those born from striped ones, which suggest the role of maternal effects mediating offspring phenotype differences between the two morphs. Female dorsal morphs and age classes differ in terms of melanin based, and brownish dorsal coloration. Adult striped and hatchling females are darker (i.e. have more melanin) than reticulated-blotched adult females and hatchlings, respectively. Moreover, adult female morphs have different morphology as reticulated-blotched females have 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 adult immune response. Finally, immune response does not differ between adult female dorsal morphs.

- Highland *P. liolepis* lizards are larger, stockier and have more femoral pores and a darker dorsal coloration than lowland ones. Thus, highland and lowland *P. liolepis* follow the same pattern of variation observed in other species within the *P. hispanicus* species complex. Dorsal and ventral coloration fluctuate seasonally and between populations. Brightness of ventral and dorsal coloration are higher in lowland than in highland lizards in spring whereas the reversed trend is found in summer. In addition, summer lizards had more throat bluish reflectance than spring ones. Males also had more throat bluish reflectance in all body regions except for the throat. We determine the existence of an anterior-posterior gradient of red ventral coloration, with the highest values in the throat. In addition, summer lizards had more brownish coloration than spring ones whereas spring lizards had more greenish coloration than summer ones.

- We confirm that the characteristics of chemical signals of male *P. hispanicus* lizards differ between two populations inhabiting environments with different climatic conditions. Lower elevation males have higher proportions of cholesterol and fatty acids, but lower proportions of alcohols than higher elevation males. Moreover, chemosensory tests with female lizards show that chemical signals of males have a lower efficacy (i.e. detectability and persistence) when time since deposition, temperature and dryness increase, but that these effects are more detrimental in the higher elevation population that occupies naturally colder and more humid environments. Therefore, interpopulational differences in chemical profiles of femoral secretions of male *P. hispanicus* lizards might reflect adaptation to maximize the efficacy of the chemical signal in different climates.

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Chapter I

This chapter reproduces entirely the manuscript:

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Altitudinally divergent adult phenotypes in Iberian wall lizards are not driven by egg differences nor hatchling growth rates

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Abstract: The interplay between ecological conditions and life histories has been widely acknowledged in vertebrates, particularly in lizards. Environmental conditions may exert different selective pressures and produce divergent phenotypes even in geographically and genetically close populations. The Iberian wall lizard, constitutes a perfect model organism as it is considered a species complex with a complicated evolutionary history. Here, we focus on two nearby populations in which we examined adult morphology and reproductive investment of wild caught lizards along a 500 m altitudinal gradient with contrasting environmental conditions, where adults show marked morphological differences in spite of being closely related. Also we performed a common garden experiment to examine embryonic and hatchling growth. We focused on reproductive investment per clutch, incubation time, egg size, morphology and growth rate of hatchlings. Results showed clutch size differences between populations that were independent of the larger body size of highland females. However, there were not egg morphological differences between populations, except for egg width, and this difference disappeared after controlling for female body size. Hatchling lizards from both populations did not differ in morphology. Moreover, we did not observe differences between populations nor sexes in hatchling growth. Overall, we provide evidence that the differences in adult body size and clutch size are not driven by size at hatching which is not contributed to by egg size, nor intrinsic hatchling growth rates associated with the environmental conditions experienced in our common garden experiment, suggesting that adult phenotypes are not the result of intrinsic differences between populations.

Keywords: Common garden, Growth, Lacertids, Phenotypic plasticity, *Podarcis hispanicus*

Introduction

A central tenet of evolutionary biology is that natural selection acting on heritable phenotypic variation will result in adaptation and differentiation among local populations inhabiting environments with different selective regimes (Edwards & Kot, 1995; Endler, 1997; Herrel *et al.,* 2006; Linhart & Grant, 1996). Life histories are a crucial part of the ecology of species and play a central role in evolutionary theory (Stearns, 2000; Du *et al.*, 2013). Life histories show variation at three levels: within populations, among populations of the same species or between species (Bauwens, 1999). This variation in life history phenotypes can be originated by different sources. In some cases, adaptive changes may be environmentally induced by extrinsic factors (phenotypic plasticity) (Losos *et al.,* 2000; Via & Lande, 1985), in others they are mediated by intrinsic differences linked to geographically variable conditions (local adaptation) (Travis, 1994); or a combination of both (Sears & Angilletta, 2003). Regardless, environmental variables are of utmost importance to understand life history variation in ectotherms, and lizards in particular (Adolph & Porter, 1993). For instance, temperature, humidity and food availability may exert different selective pressures to maximize reproductive tactics (Boyce, 1979; Bonnet *et al.,* 2001; Angilletta *et al.,* 2006). Thus, life histories vary widely between species and populations (Roff, 1992; Stearns, 1992; Du *et al.,* 2005).

As a major component of life-histories, growth patterns have profound fitness consequences through age and size at maturity and fecundity and/or adult survival (Roff, 1992; Stearns, 1992). Therefore, growth rates themselves can be honed by natural selection and adjusted to the ecological context (Adolph & Porter, 1993; Du *et al.,* 2012). Geographic variation in growth has complex sources, depending on the interplay among resource availability and the efficiency with which an organism can assimilate energy (Congdon, 1989; Ferguson & Talent, 1993; Niewiarowski & Roosenburg, 1993; Arendt, 1997).

Because these factors change with altitude, altitudinal variation in life histories, including growth rates, has been commonly studied in lizards (Niewiarowski, 2001; Caley & Schwarzkopf, 2004; Telemeco *et al.,* 2010). Most studies assume that environmental conditions (temperature and activity period) should be more restrictive as altitude increases (Olsson & Shine, 1997; Sears, 2005). However, environmental restrictions for ectotherms in Mediterranean mountains pose different problems than in temperate areas. Drought becomes less restrictive as altitude increases due to milder temperatures and higher precipitation, which increases productivity (Nahal, 1981; Iraeta *et al.,* 2012). Thus, because restrictions differ altitudinally between mediterranean and temperate areas, the study of body size variation in the mediterranean region from a life-history perspective could improve our understanding of altitudinal and latitudinal trends (e.g. Bergmann's clines).

The Iberian wall lizard, *Podarcis hispanicus* (Steindachner, 1870), is an heliothermic, insectivorous and small diurnal lacertid lizard that is variable in both morphology and life history characteristics (Pérez-Mellado, 1998). It is distributed througthout the Iberian Peninsula, and North-West Africa (Guillaume, 1987), associated to rock cliffs and boulders, and even living in close association with human constructions (Van Damme *et al.,* 1997). Recent molecular studies revealed the complex evolutionary history of *P. hispanicus*, considered as a species complex with at least five monophyletic lineages and some well recognized species, and suggest the existence of speciation within taxa previously considered to be conspecific (Guillaume, 1987; Harris & Sa-Sousa, 2002; Sá-Sousa *et al.,* 2002; Pinho *et al.,* 2007; Kaliontzopoulou *et al.,* 2011; Carretero, 2008; Renoult *et al.,* 2009; Geniez *et al.,* 2014). Even morphological analysis reveal high levels of variation both within and between mitochondrial lineages and clear diagnostic characters are lacking (Kaliontzopoulou *et al.,* 2012).

Here, we focus on two nearby *P. hispanicus* populations in which we examined adult morphology and reproductive investment of wild caught lizards. Also we report the results of a common garden experiment to examine embryonic and hatchling growth. These two populations are separated along a 500 m altitudinal gradient without clear geographical barriers but with contrasting environmental conditions. We selected these two populations because lizards differed in morphology, coloration and chemical sexual signals, in spite of being genetically closely related (Martín & López, 2006b; Gabirot *et al.,* 2012, 2013). Indeed, the two populations studied here, belong to the *P. hispanicus* type 1, which have just been named as *P. guadarramae* by Geniez *et al.,* (2014). Specifically, in these populations, lizards from higher altitude attain a larger body and head size and have more femoral pores (Gabirot *et al.,* 2012). We do not know whether this pattern of high morphological variability among populations is caused by proximate effects of local conditions or local adaptation, and this constitutes a crucial question in adaptation research (Berven & Gill, 1983). We hypothesized that, if divergent adult phenotypes are the result of either genetic and/or maternal differences among populations (intrinsic factors), we would expect size at hatching and growth rate differences under a common environment. On the other hand, if lizards plastically respond to the contrasting climatic conditions between mediterranean highland and lowland sites (extrinsic factors), as has been previously demonstrated in other lacertid lizards (Iraeta *et al.,* 2006), we would not find differences in embryonic development and size at hatching. Moreover, growth rates of hatchlings from both populations should not differ in the laboratory when controlling food, temperature and humidity in a common garden experiment.

Materials and methods

Study sites

During April-May 2011 and 2012 we captured by noosing male and gravid female lizards at two nearby populations in the Guadarrama Mountains (Central Spain). Gravidity was assessed by ventral palpation; all the females found were gravid. These population are separated 6 km by air at different altitudinal ranges. The ‘lowland’ locality is located in an oak forest (‘La Dehesa de la Golondrina´) near Cercedilla village (40º44´N, 04º02´W; 1,250 m altitude), where lizards can be found on granite rocky outcrops inside the forest. This locality has a mean annual temperature of 10.9 ºC and a mean annual rainfall of 674 mm (data from ‘Embalse de Navacerrada’ meteorological station, less than 1 km from the study site). The ‘highland’ locality is found in the upper part of a mountain valley (`Fuenfría) (40º47´N, 04º03´W; 1,750 m altitude) occupying granite rock walls and rock piles at the edge of a pine forest. In this population, mean annual temperature is 6.2 °C, and mean annual precipitation is 1170 mm (data from ‘Puerto de Navacerrada’ meteorological station, 3 km from the study site).

Adult husbandry and morphology

Immediately after capture, individuals were transported to “El Ventorrillo” field station facilities about 5 km away from the capture localities. Lizards from each population were kept separately in two identical climatic chambers (Ibercex V-450-D walk-in chambers; ASL S.A., Madrid, Spain) where temperature (diurnal = 21ºC; nocturnal = 15ºC) and photoperiod (12 h:12 h, light:dark) were easily controlled automatically. Adult lizards from the two populations were maintained separately to prevent the transmission of diseases and parasites between populations. Thus, the factors population of origin and adult husbandry chamber could not be initially partitioned. However, because gravid females were kept in the chambers just a few days (mean ± SE = 12.3 ± 0.6 days), and because the two chambers and the conditions inside were identical, we were confident that it was very unlikely that any differences between populations resulted from potential uncontrolled small differences in captivity conditions. Lizards were individually housed in plastic terraria (40x30x25 cm) filled with a moistened coconut fiber substrate and provided with a water bowl and a brick (24x11x8 cm) that allowed shelter and climbing opportunities. A 50 W halogen lamp was suspended over one end of the terrarium providing a diurnal temperature gradient (21-45 ºC) allowing thermoregulation of lizards within the preferred temperature range of this species (34.4 °C; Bauwens *et al.,* 1995). In addition, a fluorescent bulb in each shelve provided ambient lighting mimicking the natural photoperiod, and mercury vapor bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14.00 to 15.30). Adult lizards were daily watered, and fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*) *ad libitum*, dusted with a commercial vitamin and calcium supplement.

We measured body size of adult lizards using a rule (snout-vent length, ‘SVL’; to the nearest 1 mm). 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. We used general linear models (GLMs) to analyze whether these morphological variables (log-transformed) differed between population of origin and sex as fixed effects.

Reproductive investment

Cages were carefully checked for the presence of eggs twice daily. In all cases females laid eggs in the wet substrate under the brick or the water bowl. Females laid all the eggs in a single day, except one female from the lowland population that laid one egg and the other two with a difference of three days. We measured immediately 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 product of mean egg volume and clutch size. 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. Eggs 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 ºC (IRE-160; 94x60x60 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). Hence, we standardized incubation conditions to examine embryonic development as a first step in our common garden experiment during the 2011 breeding season.

When incubation was about to end, we searched daily for newborns, and the duration of incubation was noted. Laying date was referred to May 1st, and thus transformed into a continuous variable like Julian days (Warner & Shine, 2009). Of 74 females 12 laid clutches with at least one infertile egg and no female laid a clutch composed entirely of infertile eggs. Infertile clutches were discarded so only the remaining 62 females were considered in subsequent analyses. Deformed hatchlings were excluded from the analyses. Adult lizards were returned to their capture sites in late June, and juveniles released to their mothers capture sites in November prior to winter.

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 we captured them, minimizing captivity effects in our experiment. To test for differences between populations in mean egg size, clutch size, clutch mass and clutch volume, we analyzed these variables as dependent variables in general linear models (GLMs), with the population of origin as a fixed effect. To test whether there were size-independent mean egg size and clutch size differences between the populations, we also ran similar GLMs but with female body size added as a covariate.

Hatchling husbandry, morphology and growth

Immediately after hatching, juveniles were measured in a fashion identical to adults (see above) and their 'body mass' determined with a digital scale (to the nearest 0.01 g). Body condition was estimated using the residuals of the regression of log-body mass on log-SVL at hatching. Thereafter, we standardized environmental conditions in the laboratory to examine post-hatching growth in a common garden experiment. Hatchlings lizards from both populations were kept in the same climatic chamber under identical environmental conditions as adults (see above), but fed smaller prey, such as pinhead crickets (*Acheta domesticus*), fruit flies (*Drosophila hydei* and *D. melanogaster*) and small waxworms (*Galleria mellonella*). Hatchling cages were rotated along shelf rows every three days and among shelves each week to control for position effects (Telemeco *et al.,* 2010). A subset of hatchlings (n = 49) was measured again 130 days after hatching to get a rough estimate of juvenile growth prior to the onset of their first winter. 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 (size at the end/size at the beginning)/(end date−initial date)], measured in days^{-1} units (Iraeta *et al.,* 2006, 2012). Sex was assessed, and confirmed several times during the experiment, by the highly developed femoral pores in males compared to females.

We analyzed growth rates with a nested design in GLM using the statistical package Statistica 8.0 (StatSoft Inc., Tulsa, OK. USA). The mixed model tested the significance of clutch (female identity as a random factor, nested in population) to control for possible familial effects, sex and population (fixed factors) on hatchling phenotypes. It used Satterthwaite´s method of denominator synthesis and required leaving the random effects independent of the fixed effects (Searle *et al.,* 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 to analysis to meet the requirements of parametric tests.

Results

Adult morphology

Overall, highland lizards were bigger (GLM: population: $F_{1,123}$ = 46.8, $P < 0.001$; sex: $F_{1,123}$ = 8.3, $P = 0.005$; sex x population: $F_{1,123}$ = 0.1, $P = 0.709$), had more robust heads (GLMs for head length, head width and head height: population: all P´s < 0.001; sex: all P´s < 0.001; sex x population: all P´s > 0.163) and had longer femora than those from the lowland population (GLM: population: $F_{1,123}$ = 87.45, $P < 0.001$; sex: $F_{1,123}$ = 139.03, $P < 0.001$; sex x population: $F_{1,123}$ = 0.45, $P = 0.506$), with independence of sex; as it was for males compared to females in both populations. These differences were not caused by a larger body length of highland lizards, as adding SVL as a covariate did not change significance, which indicated that highland lizards, and males from both populations were also more robust (GLMs for all variables with SVL as a covariate: SVL: all P´s < 0.001; population: all *P*´s < 0.002; sex: all *P*´s < 0.001; sex x population: *P*´s > 0.094; Table 1).

Table 1. Mean (±1 SE) values for adult morphological variables in highland and lowland Podarcis hispanicus lizard populations

	Highland		Lowland	
	Females *n* = 45	Males *n* = 20	Females *n* = 45	Males *n* = 17
SVL (cm)	6.3 ± 0.1	6.7 ± 0.1	5.7 ± 0.1	5.9 ± 0.1
Head length (mm)	11.69 ± 0.13	14.28 ± 0.19	10.63 ± 0.13	12.71 ± 0.20
Head width (mm)	7.33 ± 0.08	9.19 ± 0.12	6.68 ± 0.08	8.06 ± 0.13
Head heigh (mm)	4.94 ± 0.06	6.13 ± 0.09	4.39 ± 0.06	5.23 ± 0.09
Femoral length (mm)	9.69 ± 0.12	11.98 ± 0.18	8.37 ± 0.12	10.16 ± 0.19

Reproductive investment

There were no significant differences between populations in laying dates (highland: mean + SE = 8 June ± 2.1 days; lowland: 12 June ± 2.1 days; GLM: $F_{1,60} = 2.04$, $P = 0.159$). The egg incubation period did not differ between populations (GLM: $F_{1,60} = 0.01$, $P = 0.946$; Fig. 1). Clutch size was larger in the highland than in lowland population (GLM: $F_{1,60} = 24.96$, $P <$

0.001; Table 2). However clutch size differences were not driven by the larger SVL of highland females (GLM: SVL: $F_{1,59}$ = 2.48, P = 0.121; population: $F_{1,59}$ = 5.78, P = 0.019; Table 2) despite clutch size being positively correlated with female SVL (r = 0.51, $F_{1,60}$= 20.63, P < 0.001) even after controlling for differences between populations (β = 0.57, $F_{1,59}$= 14.03, P < 0.001).

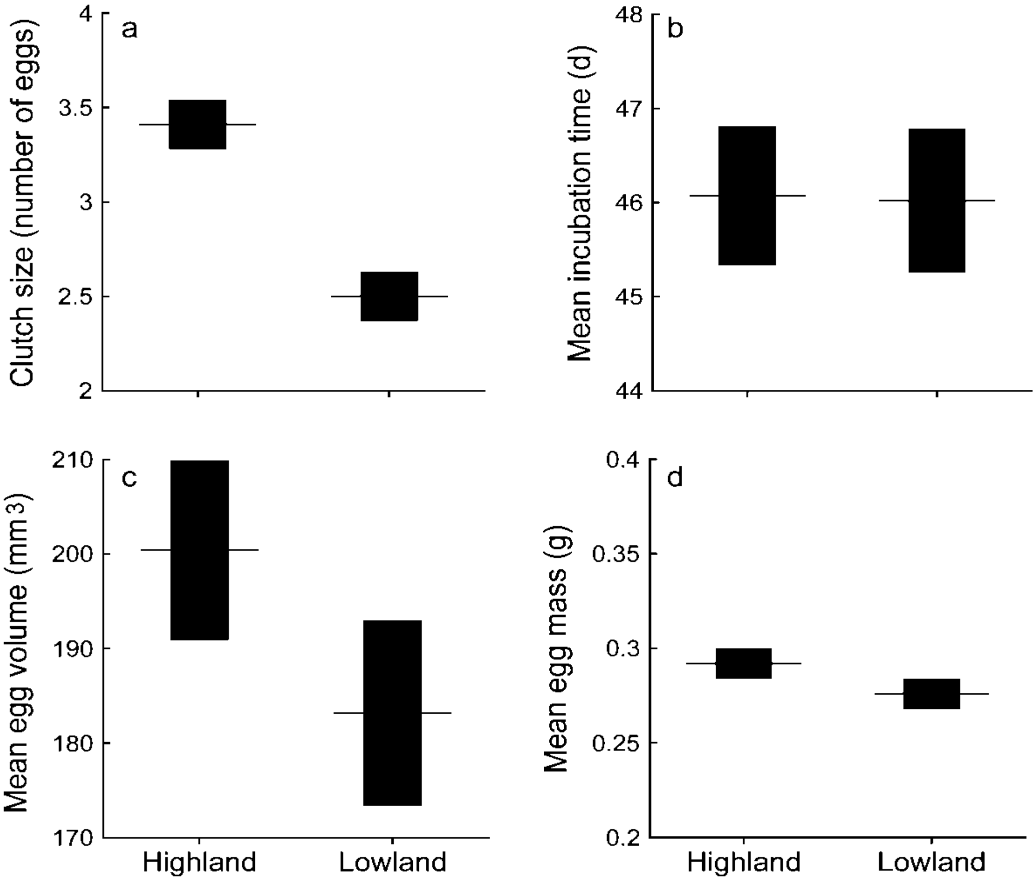

Fig. 1. Population means (±1 SE) for clutch size (a), mean incubation time (b), mean egg volume (c) and mean egg mass (d) of Podarcis hispanicus.

Clutch weight and clutch volume were greater in the highland population (GLMs: clutch weight: $F_{1,60}$ = 28.13, P < 0.001; clutch volume: $F_{1,60}$ = 20.47, P < 0.001). Both clutch weight and clutch volume remained different between populations after controlling for female SVL (GLMs: clutch weight: SVL: $F_{1,59}$ = 5.64, P = 0.021; population; $F_{1,59}$= 4.59, P = 0.036; clutch volume: SVL: $F_{1,59}$ = 1.12, P = 0.294; population: $F_{1,59}$ = 15.36, P < 0.001; Table 2).

Mean egg length did not differ between populations (GLM: $F_{1,60} = 0.51$, $P = 0.478$; Table 2) but the eggs of highland lizards were wider (GLM: $F_{1,60} = 4.09$, $P = 0.048$; Table 2), which seemed to be a direct effect of a large female body size in this population (GLM: SVL: $F_{1,59} = 2.50$, $P = 0.119$; population: $F_{1,59} = 0.09$, $P = 0.769$). Overall, there were not significant differences between populations in mean egg mass (GLM: $F_{1,60} = 2.12$, $P = 0.150$; Fig. 1) or mean egg volume (GLM: $F_{1,60} = 2.00$, $P = 0.162$; Fig. 1).

Table 2 Mean (±1 SE) values for clutch and hatchling characteristics in highland and lowland Podarcis hispanicus lizard populations (n = number of clutches).

	Highland ($n = 32$)	Lowland ($n = 30$)
Eggs:		
Clutch weight (g)	0.99 ± 0.04	0.69 ± 0.04
Clutch volume (mm^3)	688.54 ± 37.51	450.68 ± 38.74
Egg length (mm)	11.15 ± 0.17	11.34 ± 0.18
Egg width (mm)	6.72 ± 0.08	6.49 ± 0.08
Hatchlings:		
Mass (g)	0.39 ± 0.01	0.38 ± 0.01
SVL (cm)	2.86 ± 0.04	2.90 ± 0.04
Body condition (g cm^{-1})	0.011 ± 0.008	-0.011 ± 0.008
Head length (mm)	7.07 ± 0.05	7.10 ± 0.05
Head width (mm)	4.00 ± 0.03	4.01 ± 0.03
Head height (mm)	2.87 ± 0.02	2.79 ± 0.02
Femoral length (mm)	4.36 ± 0.06	4.41 ± 0.07

Hatchling morphology and growth

Lizards from both populations showed a high hatching rate (lowland: 90.7%; highland: 91.7%). Deformed newborns were extremely rare (three newborns from the lowland and two from the highland site). Deformities were localized in the tail (shorter with a kinked or curved tip) and/or the hands and feet, affecting finger or toe number and position.

Hatchling lizards from both populations were morphologically similar in body size (GLMs on hatchling SVL; population: $F_{1,76} = 1.38$, $P = 0.244$; sex: $F_{1,76} = 17.87$, $P < 0.001$; population x sex: $F_{1,76} = 0.27$, $P = 0.606$; see clutch averaged values in Table 2), body mass (GLM: population: $F_{1,76} = 2.03$, $P = 0.160$; sex: $F_{1,76} = 1.61$, $P = 0.209$; population x sex: $F_{1,76} = 0.60$, $P = 0.441$), body condition (GLM: population: $F_{1,76} = 0.89$, $P = 0.349$; sex: $F_{1,76} = 5.55$, $P = 0.021$; population x sex: $F_{1,76} = 2.79$, $P = 0.099$), head shape (GLMs for head length, head width and head height; population: all P′s > 0.053; sex: all P′s > 0.155; sex x population: all P′s > 0.304) and femoral length (GLM: population: $F_{1,76} = 0.28$, $P = 0.601$; sex: $F_{1,76} = 4.28$, $P = 0.042$; population x sex: $F_{1,76} = 0.33$, $P = 0.253$). Hatchling females were larger and had longer femora but lower body condition than males (see the previous GLMs). However, after controlling for hatchling SVL, males had relatively longer and wider heads (GLM: head length: SVL: $F_{1,75} = 119.28$, $P < 0.001$; population: $F_{1,75} = 0.52$, $P = 0.473$; sex: $F_{1,75} = 16.25$, $P < 0.001$; population x sex: $F_{1,75} = 0.89$, $P = 0.350$; head width: SVL: $F_{1,75} = 122.33$, $P < 0.001$; population: $F_{1,75} = 0.83$, $P = 0.365$; sex: $F_{1,75} = 7.52$, $P = 0.008$; population x sex: $F_{1,75} = 0.79$, $P = 0.376$) while relative head height was similar between sexes (GLM: SVL: $F_{1,75} = 33.86$, $P < 0.001$; population: $F_{1,75} = 2.43$, $P = 0.123$; sex: $F_{1,75} = 3.61$, $P = 0.061$; population x sex: $F_{1,75} = 2.50$, $P = 0.118$).

A mixed model GLM of the different growth variables (see Table 3), with clutch as a random factor and population and sex as fixed factors did not show significant differences between populations in mass-specific growth, size specific growth, head growth or femoral growth, nor between sexes (see Table 4). There were not sex and population body condition significant differences at the end of the experiment (GLM: population: $F_{1,45} = 0.05$, $P = 0.826$; sex: $F_{1,45} = 0.02$, $P = 0.889$; population x sex: $F_{1,45} = 0.27$, $P = 0.107$).

Table 3. Mean (±1 SE) growth rates (days^{-1}) for the morphological variables measured, for hatchlings from two populations of Podarcis hispanicus lizards reared under a common garden experiment.

	Highland		Lowland	
	Females	Males	Females	Males
	n =14	*n* =13	*n* =12	*n* =10
Mass-specific growth (days^{-1})	-0.00371 ± 0.00081	-0.00331 ± 0.00084	-0.00334 ± 0.00087	-0.00399 ± 0.00095
Size-specific growth (days^{-1})	0.00053 ± 0.00051	0.00043 ± 0.00052	0.00036 ± 0.00055	0.00120 ± 0.00060
Head length growth (days^{-1})	0.00342 ± 0.00064	0.00354 ± 0.00067	0.00278 ± 0.00069	0.00362 ± 0.00076
Head width growth (days^{-1})	0.00297 ± 0.00044	0.00336 ± 0.00046	0.00316 ± 0.00048	0.00294 ± 0.00053
Head heigth growth (days^{-1})	-0.00055 ± 0.00073	-0.00048 ± 0.00075	0.00016 ± 0.00079	-0.00056 ± 0.00086
Femoral growth (days^{-1})	0.00604 ± 0.00037	0.00633 ± 0.00039	0.00631 ± 0.00040	0.00668 ± 0.00044

Table 4. Effect of population, sex, clutch and; population x sex and sex x clutch interactions (mixed model GLM) on growth for six morphological variables of hatchlings from two populations of Podarcis hispanicus lizards reared under a common garden experiment

	Population	Sex	Clutch	Population x sex
Mass-specific growth	$F_{1,31.27} = 1.55$	$F_{1,19.00} = 0.07$	$F_{26,19.00} = 2.46$	$F_{1,19.00} = 0.99$
	$P = 0.222$	$P = 0.797$	$P = 0.024$	$P = 0.333$
Size-specific growth	$F_{1,31.80} = 0.41$	$F_{1,19.00} = 0.04$	$F_{26,19.00} = 2.22$	$F_{1,19.00} = 0.01$
	$P = 0.526$	$P = 0.847$	$P = 0.038$	$P = 0.975$
Head length growth	$F_{1,30.88} = 1.58$	$F_{1,19.00} = 0.13$	$F_{26,19.00} = 2.67$	$F_{1,19.00} = 0.11$
	$P = 0.218$	$P = 0.718$	$P = 0.016$	$P = 0.742$
Head width growth	$F_{1,36.05} = 0.71$	$F_{1,19.00} = 0.06$	$F_{26,19.00} = 1.21$	$F_{1,19.00} = 0.75$
	$P = 0.404$	$P = 0.807$	$P = 0.341$	$P = 0.398$
Head heigth growth	$F_{1,36.02} = 0.15$	$F_{1,19.00} = 0.19$	$F_{26,19.00} = 1.21$	$F_{1,19.00} = 0.95$
	$P = 0.704$	$P = 0.664$	$P = 0.338$	$P = 0.343$
Femoral length growth	$F_{1,32.04} = 0.01$	$F_{1,19.00} = 0.14$	$F_{26,19.00} = 2.13$	$F_{1,19.00} = 0.53$
	$P = 0.964$	$P = 0.708$	$P = 0.047$	$P = 0.475$

A significant effect of clutch was found for mass-specific growth, size-specific growth, head length growth and femoral growth (Table 4). This clutch effect only disappeared after controlling for relative clutch size (mixed model GLM: clutch: $F_{26,\ 18.00} = 1.92$, $P = 0.078$) and egg weight (mixed model GLM: clutch: $F_{26,18.00} = 2.02$, $P = 0.063$) in the case of femoral growth. The rest of growth variables remained significant after controlling for both effects (all *P´s* < 0.05). However the relationships between femoral growth and relative clutch

($r = 0.24$, $F_{1.47} = 2.87$, $P = 0.097$) and egg weight ($r = 0.06$, $F_{1.47} = 0.15$, $P = 0.701$) were not clear.

Discussion

Our study provides clear evidence that altitudinal divergent adult body size in two proximate populations of Iberian wall lizards is not caused by contrasting reproductive investment per egg nor different pre- and post-hatching growth rates. High altitude adult lizards were larger with a robust head morphology and had longer femora. Hence, our lizard populations follow Bergmann´s rule (i.e. larger body size in colder environments). This result is in sharp contrast with the vast mayority of squamates which exhibit reversed Bergmann´s clines (Ashton & Feldman, 2003). However, different trends can be found within a genus (Ashton, 2003; Sears, 2005) or even within the same species (e.g. *Sceloporus undulatus*; Ferguson & Talent, 1993; Niewiarowski & Roosenburg, 1993). Geographical variation in body size can be produced by mechanisms not necessarily related to bergmann´s rule (Palkovacs, 2003) and a life-history perspective can enrich our understanding of Bergmann´s clines (Roff, 1980; 1986). In this context, we did not find differences in growth between high and low altitude hatchling lizards or between sexes in our common garden experiment. Thus, our results agree with those studies where growth differences among populations and adult body size are likely a plastic response and not a product of intrinsic differences (Niewiarowski & Roosenburg, 1993; Sorci *et al.,* 1996, Lorenzon *et al.,* 2001). Deviations from the reversed Bergmann´s size clines in squamates are likely to arise by two different processes: environmental limitation of growth in warmer climates (through resource limitation or process limitation; Congdon, 1989; Dunham *et al.,* 1989) or selection for a specific body size or growth rate via age specific mortality (Stearns & Koella, 1986).

Resource limitation exists when an individual cannot acquire sufficient resources from the environment to support maximal growth. One form of resource limitation is process limitation (Congdon, 1989). Even when food availability is high, lizards may not take advantage of it due to thermal constraints on ingestion and assimilation. This has been described in the lizard *Sceloporus merriami*, where the prolonged exposure to night-time cool temperatures limits the rate at which food items pass through the gut (Dunham *et al.,* 1989). However, this possibility alone is in contrast to our findings of highland *P. hispanicus* attaining a larger body size. Appart from behavioural interferences of environmental origin, such as predation risk, a common cause of resource limitation is produced when material resources (e.g. food or water) are limited in the environment (Dunham, 1978). Several studies support a link between growth rates and habitat production via climate effects (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin, 1985). If these effects persist over time, they could generate differential body size and life-histories among populations like those reported here (Buckley *et al.,* 2007; Díaz *et al.,* 2011). That would imply two alternative mechanisms to explain the absence of an origin effect in growth under our common garden experiment. First, environmental constraints such as thermoregulation opportunities or food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability (Stamps & Tanaka, 1981). Second, growth plasticity itself (physiologically) could not be adaptive, it may be the consequence of adaptive plasticity at the behavioural level enabling lizards to avoid dehydration (Jones *et al.,* 1987; Lorenzon *et al.,* 1999).

Our study cannot tease apart the intrinsic and extrinsic factors that shape reproductive investment differences but, as clutch size, clutch volume and clutch weight were shown to be independent of the larger size of highland females, it is interesting to speculate how environmental conditions could potentially drive these differences. As with body size, there is growing evidence that this pattern of variation in reproductive traits (i.e. larger lizards laying big clutches) may be caused by environmental differences in productivity. Tropidurid lizards show a positive relationship of clutch and female body size with precipitation, possibly via effects on primary production (Brandt & Navas, 2011). In the lacertid lizard *Psammodromus algirus*, female body size and clutch size were larger and positively associated with a gradient of increasing humidity and decreasing temperature (Díaz *et al.,* 2011). Similarly, female *P. hispanicus* from our highland population laid larger clutches of wider eggs. Iraeta *et al.,* (2006) showed that food availability was higher in a high altitude site than in a low altitude site in the same geographical area of our study. Also, preliminary surveys indicate that food availability is higher in our highland population in early spring (unpubl. data). Moreover, in late September, at the end of the breeding season, when summer drought was more evident, soil humidity was still high and lizards and insects were still abundant at the highland site; whereas at the lowland site, the soil was completely dry and lizards and their prey were very rarely observed (unpubl. data). This pattern seems likely in Mediterranean lowlands where precipitation, primary production, and hence food availability for insectivores tend to be higher in mountain ranges than in the surrounding xeric lowlands, allowing lizards to grow to larger sizes (Iraeta *et al.,* 2006). In addition, higher productivity and reproductive investment are tightly linked to high precipitation (Jordan & Snell, 2002). Female *P. hispanicus* may lay several clutches during the breeding season (Galan, 2003) so it remains unknown if the annual total reproductive effort of these lowland and highland populations differ. In line with this, Aragón & Fitze (2014) acknowledged primary productivity as an important predictor of body size in another lacertid, the Spanish sand racer; and suggested how higher juvenile survival due to higher food availability might outweigh the costs of delayed maturation, or higher growth rates due to a higher food intake might counteract growth retardation at lower temperatures.

Classical models of life history evolution predict that when juvenile mortality is high, fast growth is favored (Stearns, 1992, 2000). Hence, Bergmann size clines can be produced by mortality schedules that favor earlier reproduction in warmer environments than in cooler environments (Sears & Angiletta, 2004). Often, fast growth incurs the cost of maturing at a relatively smaller adult body size. Because longer potential periods of activity, such as those found in warmer environments, can produce higher rates of mortality (Wilson, 1991), selection may produce faster growing lizards that achieve smaller adult body sizes, resulting in a Bergmann size cline. However our results do not offer any evidence to support selection for a specific body size or growth rate via age specific mortality as we did not find growth differences under a common garden experiment. Large body size results from either faster growth, a longer duration of growth, or both (Angilletta *et al.,* 2004). Since ectotherms grow slower at lower temperatures, prolonged growth and delayed maturation have been invoked as the proximate mechanism for a larger body size in colder environments (Atkinson, 1994; Morrison & Hero, 2003). In keeping with this, survival tends to be higher in colder environments (than in warmer lowlands, for instance) in some lizard species which delay maturation until reaching larger body sizes (Angilletta *et al.,* 2004). In addition, individuals with longer activity season are therefore expected to grow faster and attain maturation at smaller size and earlier age (Adolph & Porter, 1993; Sears, 2005). For example, length of activity season, and not temperature per se, is the main environmental factor constraining growth rate and annual rates of mortality in *Zootoca vivipara* (Horváthová *et al.,* 2013). Thus, the relative contribution of activity period and survival to explain the adult body size differences observed in our lizard populations should be further explored.

In conclusion, the work presented here is the first attempt to experimentally unravel the causes of morphological variation within the *P. hispanicus* species complex. We provide clear evidence that the divergent adult body sizes are not driven by size at hatching, which is not contributed to by egg size, nor intrinsic post-hatching growth rates associated with the environmental conditions experienced in our common garden experiment. Thus our results suggest that adult phenotypes are not the result of intrinsic differences but the consequence of a plastic response. Further research should aim to determine which factors could drive this phenotypic plasticity. Environmental conditions and their effects on primary production, activity period or survival, could play a key role in maintaining phenotypic divergence. The relative contribution of phenotypic plasticity and local adaptation to different climatic conditions remain to be assessed by an ongoing reciprocal transplant experiment (unpubl. data). More research is needed to clarify the role of phenotypic plasticity as a trigger of divergence within and among populations or as a buffer against it. Because an evolutionary change in body size can be regarded as an important component of the speciation processes (Sistrom *et al.,* 2012), we propose the *P. hispanicus* species complex as a good model to investigate the relative importance of phenotypic plasticity along the speciation continuum.

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Chapter II

Environmental–determined growth may explain altitudinal body size differences in Iberian wall lizards: a reciprocal transplant experiment

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Abstract: Geographic variation in growth has complex sources, depending on the interplay among resource availability and the efficiency with which an organism can assimilate energy. Thus, the interpretation of interpopulation differences in growth rates along geographical gradients requires distinguishing between the proximal (environmental) and ultimate (genetic) causes of growth. Several studies support a link between growth rates and habitat production via climate effects. So environmental constraints such as thermoregulation opportunities or food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability. Here we perform a reciprocal transplant experiment to examine growth rates between two closely related populations of *P. hispanicus* along a 500-m altitudinal gradient with contrasting environmental conditions. Our results showed that the growing environment determined mass-specific and size-specific growth rates, independently of population of origin. In addition, the drivers of growth rate differences were likely between-sites differences in abundance and the diversity of arthropods (i.e. food availability and quality) as a result of a higher ecosystem productivity in the highland site.

Keywords: Reciprocal transplant, productivity, food availability, growth, body size

Introduction

Life histories are a crucial part of the ecology of species and play a central role in evolutionary theory (Stearns, 2000; Du *et al.,* 2013). Studies of intraspecific variation in life-histories are typically concerned with identifying environmental sources of variation that are correlated with observed variation (Tinkle & Ballinger, 1972, Ballinger, 1977, Jones & Ballinger, 1987, Ford & Siegel, 1989). In some cases, variation in life-history traits may be environmentally induced by extrinsic factors (phenotypic plasticity) (Losos *et al.,* 2000; Via & Lande, 1985), in others they are mediated by intrinsic differences linked to geographically variable conditions (local adaptation) (Travis, 1994); or a combination of both (Sears & Angilletta, 2003). Hence, the understanding of the ecological and evolutionary significance of intraspecific geographic variation in life history traits requires teasing apart genetic and environmental sources of variation (Berven *et al.,* 1979; Ferguson & Brockman, 1980; Ballinger, 1983; Berven & Gill, 1983; Jones & Ballinger, 1987; Ford & Siegel, 1989).

The optimal life history strategy for a species has been defined as the one that maximizes lifetime reproduction, which is determined by maximizing age-specific survival and fecundity (Roff, 1992; Stearns, 1992). For the vast majority of species, however, it is size rather than age that determines both survival probabilities (Gross, 1981; Werner, 1988) and fecundity (Shine, 1988).(Dunham, 1978; Siegel & Ford 1991; Sinervo & Adolph, 1994; Lorenzon *et al.,* 2001). Geographic variation in growth has complex sources, depending on the interplay among resource availability and the efficiency with which an organism can assimilate energy (Congdon, 1989; Ferguson & Talent 1993; Niewiarowski & Roosenburg, 1993; Arendt, 1997). Thus, the interpretation of interpopulation differences in growth rates along geographical gradients requires distinguishing between the proximal (environmental) and ultimate (genetic) causes of growth (Iraeta *et al.,* 2006).

Most of the studies assume that environmental conditions, such as temperature and humidity, and other factors that determine the activity period, should be more restrictive as altitude increases so the time available for hatchling growth prior to winter is more limited than at lower altitude (Olsson & Shine, 1997; Sears, 2005). However, environmental restrictions for ectotherms differ altitudinally between mediterranean and temperate areas. In the former, drought becomes less restrictive as altitude increases due to milder temperatures and higher precipitation, which increases productivity (Nahal, 1981; Iraeta *et al.,* 2012). Several studies support a link between growth rates and habitat production via climate effects (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin, 1985; Aragón & Fitze, 2014). So environmental constraints such as thermoregulation opportunities or food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability (Ballinger, 1977; Dunham, 1978; Ballinger & Congdon, 1980; Stamps & Tanaka, 1981). Hence, the main problem for ectotherms in Mediterranean lowlands during the dry season (i.e. summer) may be food scarcity rather than thermal constraints, where offspring may experience reduced growth due to food shortage (Iraeta *et al.,* 2006).

Results from a common garden experiment provided evidence that the divergent adult body sizes of *P. hispanicus* lizards are not driven by size at hatching, which is not contributed to by egg size, nor intrinsic post-hatching growth rates (Ortega *et al.,* 2015). Overall, our results suggest that adult phenotypes are not the result of intrinsic differences but the consequence of a plastic response. The work presented here constitutes the next logical step, and aims to determine which factors drive phenotypic plasticity in this species. Thus, we performed a reciprocal transplant experiment of hatchling lizards to determine the relative contribution of phenotypic plasticity and local adaptation to different climatic conditions. In this study we compare hatchling growth rates and food availability in two nearby populations of *P. hispanicus* lizards, in the Guadarrama Mountains. These two populations are separated along a 500 m altitudinal gradient without clear geographical barriers but with contrasting environmental conditions. We selected these two populations because lizards differed in adult body size, morphology, coloration and chemical sexual signals, in spite of being genetically closely related (Martin & López, 2006; Gabirot *et al.,* 2012, 201 Specifically, we hypothesized that if climatic differences were responsible for growth rate differences, then source population would not determine growth rate and, hence, we would find the same phenotypes in the same environment. Conversely, if phenotypic divergence between populations is maintained across different environments, genetic differences would be responsible for juvenile growth rates. We also measured food availability in both populations to test the hypothesis that productivity (i.e. food availability) has a direct effect on lizard growth rates. We predicted that lizards would grow faster in the site with higher precipitation levels and, ultimately, higher ecosystem productivity.

Material and methods

Study sites

During April-May 2013 gravid female *P. hispanicus* lizards were captured by noosing at two nearby populations in the Guadarrama Mountains (Central Spain). The lowland locality was `La Dehesa de la Golondrina´, an oak forest near Cercedilla village (40º44´N, 04º02´W; 1,250 m altitude). The ‘highland’ locality is found in the upper part of a mountain valley (`Fuenfría) (40º47´N, 04º03´W; 1,750 m altitude) occupying granite rock walls and rock piles at the edge of a pine forest. These populations are located at different altitudinal ranges with contrasting climatic conditions separated 6 km by air (Ortega et al., 2015) Microsatellite analyses show that lizards from these two populations are closely related genetically, indicating that are two sampling localities of the same genetic subpopulation (Gabirot *et al.,* 2013).

Adult husbandry

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 one climatic room where temperature (diurnal = 21ºC; nocturnal = 15ºC) and photoperiod (12 h: 12 h, light: dark) were easily controlled automatically. Lizards were individually housed in plastic terraria (40x30x25 cm) filled with a moistened coconut fiber substrate and provided with a water bowl and a brick (24x11x8 cm) that allowed shelter and climbing opportunities. A 50 W halogen lamp (hotspot) was suspended over one end of the terrarium providing a diurnal temperature gradient (21–45 ºC) allowing thermoregulation of lizards within the preferred temperature range of this species (34.4 °C; Bauwens *et al.,* 1995). In addition, fluorescent bulbs over the terraria provided ambient lighting mimicking the natural photoperiod, and mercury vapor bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14.00 to 15.30). Adult lizards were daily watered, and fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*) *ad libitum*, dusted with a commercial vitamin and calcium supplement. Adult lizards were returned to their capture sites in late June.

Eggs and hatchlings husbandry

Cages were carefully checked for the presence of eggs twice daily. 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 thus captivity effects in our experiment. Eggs 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) where they were randomly distributed and shelves were also rotated each week to control for possible position effects (Telemeco *et al.,* 2010). Immediately after hatching, we measured body size using a ruler [snout-vent length (SVL) to the nearest 1 mm]. We assessed 'body mass' with a digital scale (to the nearest 0.01 g).

Preliminary studies revealed high hatchling mortality during the winter in natural conditions (J. Ortega unpubl. results). Thus, we decided to keep newborns in the laboratory under standardized environmental conditions until the next spring, as we did in a previous common garden experiment (Ortega *et al.,* 2015). From December to February, we simulated an invernal pause, reducing ambient temperature below 10 °C and the availability of a hotspot to only 1.5 hours a day. As this species is active all the year round and does not stand a strict hibernation (J. Ortega unpubl. data) the former procedure was suspended for 3 days per month where ambient temperature was raised above 15 °C and the hotspot available for 5 hours. Insects of an appropriate size were offered *ad libitum* the first day of each of the winter breaks.

Field procedures

Four outdoor enclosures (5 x 5 m^2) , two per locality, were built in the typical habitat were lizards are found without modifying the natural vegetation cover and the surroundings. Galvanized metal walls of 85 cm height, partly buried in the floor (25 cm) delimited each enclosure. A net covered the enclosures from above to avoid bird predation. Five artificial boulders per enclosure, each one composed of four concrete bricks (50 x 25 x 25 cm) and covered with granite rocks, were added to mimic the saxicolous habitat of the species. Under each boulder we excavated a pit of 25 cm of depth to fit a clay brick (40 x 30 x 25 cm) with 6 longitudinal holes to provide additional underground shelter.

Hatchlings were toe-clipped and photographed for further identification and released in the outdoor enclosures in spring (2nd May) following a split clutch design. Hatchlings from a given clutch were randomly assigned to the release plots at the low-elevation site (n = 26), and t to the high-elevation site (n = 26) so that at least 2 hatchlings per female were raised in the two treatments.

After release, hatchlings were measured again (see above) on late spring (6th- 8th June) and late summer (9th- 11st September) to estimate juvenile growth during the activity period. We searched for juveniles below bricks and rocks around 9:00 am, before they became active. We repeated this sampling for three days in every sampling period to recapture all or most of lizards. Lizards were identified, measured, weighed and released again in the enclosures immediately after capture.

To estimate food availability and prey diversity, we walked arbitrary transects at each study site by tossing a 20 × 20 cm^2 wooden frame 24 times and counting and identifying all arthropods contained within it during 1 min of search (Díaz & Carrascal 1990). As *P. hispanicus* is a saxicolous species we concentrated our sampling on rock boulders, rock cliffs and their surroundings. All prey items were identified to order except ants. Food availability was estimated matching the timing of hatchling release and the two recapture periods. As *P. hispanicus* is a generalist species (Salvador & Carretero 2014) the availability of arthropods mirrors the potential prey availability. Diversity of arthropods was calculated by means of the Shannon–Weaver index ($H'=-\Sigma pi \ln pi$) for the taxonomic categories identified (Magurran, 1988).

Statistical analysis

The size-specific (SVL) and mass-specific growth 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 days^{-1} units (Iraeta *et al.,* 2006, 2012). To control for possible familial effects, we also compared the size-specific growth rates of half-siblings released at different sites using repeated-measures GLM. Body condition was estimated using the residuals of the regression of log–body mass on log-SVL. Except for the third recapture period, where we performed non-parametric analyses, all the statistical analysis were performed with general linear models and mother origin and release sites as fixed factors (GLM) and were conducted in Statistica 8.0 (StatSoft Inc., Tulsa, OK. USA).

Results

Food availability

The number of available potential prey items was significantly higher in the highland site and there were significant differences between seasons in both populations (GLM: season: $F_{2,138}$ = 13.16, $P < 0.001$; population $F_{1,138}$ = 90.15, $P < 0.001$; season x population: $F_{2,138}$ = 1.53, P = 0.220). Tukey´s post hoc tests revealed that prey availability was lower in summer than in early spring (P = 0.007) and late spring ($P < 0.001$), while there were no significant differences between the two spring sampling periods (P = 0.095). The diversity of arthropods differed between seasons in both populations and although the diversity of arthropods tended to be higher in the highland site the difference did not reach significance (GLM: season: $F_{2,138}$ = 3.59, P = 0.030; population $F_{1,138}$ = 3.20, P = 0.076; season x population: $F_{2,138}$ = 0.34, P =

0.713). Tukey´s post hoc tests revealed significant differences between late spring and summer (*P* = 0.035), while early spring did not significantly differ from late spring (*P* = 0.129) or summer (*P* = 0.888).

Hatchling morphology

Body size, body mass and the body condition of hatchlings from both populations was similar at hatching in the laboratory (GLMs: SVL: $F_{1,50}$ = 1.50, *P* = 0.218; body mass: $F_{1,50}$ = 2.59, *P* = 0.114; body condition: $F_{1,50}$ = 0.06, *P* = 0.808) (highland population: SVL: 3.18 ± 0.03 cm; body mass: 0.42 ± 0.01 g; body condition: 0.003 ± 0.008 g cm^{-1}; lowland population :SVL: 3.15 ± 0.03 cm; body mass: 0.40 ± 0.01 g; body condition: -0.003 ± 0.008 g cm^{-1}).

Table 1. Mean (±1 SE) values for hatchling morphological variables depending on release site and population of origin in Podarcis hispanicus lizards.

	Late spring				Summer			
	Population origin		Release site		Population origin		Release site	
	Highland	Lowland	Highland	Lowland	Highland	Lowland	Highland	Lowland
SVL (cm)	5.18 ± 0.11	5.33 ± 0.12	5.57 ± 0.09	4.93 ± 0.09	6.2 ± 0.2	6.5 ± 0.3	6.7 ± 0.1	5.8 ± 0.2
Body mass (g)	1.29 ± 0.06	1.42 ± 0.07	1.50 ± 0.05	1.20 ± 0.05	3.9 ± 0.3	4.2 ± 0.4	4.7 ± 0.1	3.2 ± 0.1
Body condition (g*cm^{-1})	0.001± 0.013	0.001 ± 0.014	0.006 ± 0.013	-0.006 ± 0.014	0.005 ± 0.006	0.008 ± 0.007	-0.002± 0.007	0.003 ± 0.007

The absence of body size, body mass and body condition differences between hatchlings remained at the moment of release in May (GLMs: SVL: $F_{1,50} = 1.50$, $P = 0.218$; body mass: $F_{1,50} = 2.59$, $P = 0.114$; body condition: $F_{1,50} = 0.01$, $P = 0.99$) (highland population: SVL: 3.59 ± 0.04 cm; body mass: 0.63 ± 0.03 g; body condition: -0.008 ± 0.011 g cm^{-1}; lowland population :SVL: 3.67 ± 0.04 cm; body mass: 0.69 ± 0.03 g; body condition: 0.008 ± 0.011 g cm^{-1}). We confirmed that body size, body mass and body condition did not differ between enclosures (all *P´s* > 0.323).

Table 2. Mean (±1 SE) values for hatchling growth rates in two recapture periods depending on release site and population of origin in Podarcis hispanicus lizards.

	Late spring		Summer	
	Size-specific growth rate ($days^{-1}$)	Mass-specific growth rate ($days^{-1}$)	Size-specific growth rate ($days^{-1}$)	Mass-specific growth rate ($days^{-1}$)
Population origin				
Highland	0.04725 ± 0.00108	-0.00734 ± 0.00123	-0.005080 ± 0.005696	-0.005080 ± 0.005696
Lowland	0.04852 ± 0.00116	-0.00813± 0.00133	0.008129 ± 0.007205	0.008129 ± 0.007205
Release site				
Highland	0.05049 ± 0.00110	0.00382 ± 0.00126	0.004458 ± 0.000459	0.013450 ± 0.000681
Lowland	0.04528 ± 0.00113	-0.01166 ± 0.00129	0.001468 ± 0.00495	0.008348 ± 0.000736

A GLM with recaptured lizards in June (36 ± 1 days after they had been released) with mother´s origin and release site as factors (release site: $N = 20$; highland; $N = 19$; population of origin: lowland; $N = 21$; highland; $N = 18$; lowland) showed that lizards were larger and heavier in the high elevation enclosures with independence of the population of origin (SVL: GLM, release site: $F_{1,35} = 28.70$, $P < 0.001$; site of origin: $F_{1,35} = 1.83$, $P = 0.185$; release site x site of origin: $F_{1,35} = 0.42$, $P = 0.523$; Body mass: GLM, release site: $F_{1,35} = 18.33$, $P < 0.001$; site of origin: $F_{1,35} = 2.71$, $P = 0.109$; release site x site of origin: $F_{1,35} = 0.07$, $P = 0.794$). However, body condition was similar between release sites and population of origin

(GLM: release site: $F_{1,35} = 0.34$, $P = 0.560$; site of origin: $F_{1,35} = 0.74$, $P = 0.394$; release site x site of origin: $F_{1,35} = 0.06$, $P = 0.804$) (see Table 1).

Lizards recaptured in September were also larger ($N = 7$, highland; $N = 6$, lowland; Mann–Whitney's U-test: $U = 2.00$, $Z = -2.71$, $P = 0.007$) and heavier in the high elevation enclosures ($N = 7$, highland; $N = 6$, lowland; Mann–Whitney's U-test: $U = 0.00$, $Z = -3.00$, $P = 0.003$). The population of origin did not affect body size ($N = 8$, highland; $N = 5$; lowland; Mann–Whitney's U-test: U = 13.50, Z = -0.95, P = 0.341) or body mass ($N = 8$, highland: 3.9 ± 0.3 g, $N = 5$; lowland: 4.2 ± 0.4 g, Mann–Whitney's U-test: U = 20.00, $Z = 0.00$, $P = 1.000$). However, body condition did not differ between release sites ($N = 7$; highland; $N = 6$; lowland; Mann–Whitney's U-test: U = 21.00, Z = 0.00, P = 1.00) or population of origin ($N = 8$; highland; $N = 5$; lowland; Mann–Whitney's U-test: $U = 8.00$, $Z = -1.76$ $P = 0.079$) (see Table 1).

Growth

We found differences in size-specific (release site: $N = 20$; highland; $N = 19$; population of origin: lowland; $N = 21$; highland; $N = 18$; lowland) (GLM: release site: $F_{1,35} = 10.84$, $P = 0.002$; site of origin: $F_{1,35} = 0.64$, $P = 0.428$; release site x site of origin: $F_{1,35} = 0.02$, $P = 0.902$) and mass–specific growth (GLM: release site: $F_{1,35} = 10.93$, $P < 0.001$; site of origin: $F_{1,35} = 0.19$, $P = 0.663$; release site x site of origin: $F_{1,35} = 0.17$, $P = 0.682$) due to release site but not for population of origin in June. Thus hatchlings released in the highland site increased and body mass faster than those released in the lowland site, or between population of origin (see Table 2).

A repeated measures GLM comparing the (average) growth rates of half-sibs reared at both sites showed that size-specific growth rates were consistently higher at the high-elevation site (repeated measures GLM: release site: $F_{1,14} = 64.32$, $P < 0.001$; site of origin: $F_{1,14} = 0.26$, $P = 0.620$; release site x site of origin: $F_{1,14} = 1.09$, $P = 0.315$). The same result was true for mass-specific growth (repeated measures GLM: release site: $F_{1,14} = 22.52$, $P = 0.003$; site of origin: $F_{1,14} = 0.31$, $P = 0.587$; release site x site of origin: $F_{1,14} = 0.01$, $P = 0.909$).

Lizards recaptured in September showed a higher size-specific growth rate ($N = 7$; highland; $N = 6$; lowland; Mann–Whitney's U-test: $U = 0.00$, $Z = -3.00$, $P = 0.003$) and a higher mass-specific growth rate in the highland than in the lowland enclosures ($N = 7$; highland; $N = 6$; lowland; Mann–Whitney's U-test: $U = 1.00$, $Z = -2.86$, $P = 0.004$). However we did not detect differences due to population of origin in size-specific growth ($N = 8$; highland; $N = 5$; lowland; Mann–Whitney's U-test: $U = 19.00$, $Z = 0.15$, $P = 0.884$) or mass-specific growth ($N = 8$; highland; $N = 5$; lowland; Mann–Whitney's U-test: $U = 12.00$, $Z = 1.17$, $P = 0.242$). (see Table 2).

Discussion

Our results indicated that lizards grew faster in the higher altitude environment, with independence of their population of origin. Thus, this study confirms our previous interpretation that growth differences and adult body size are a plastic response and not a product of intrinsic differences among populations (Ortega *et al.,* 2015). High altitude adult lizards are larger that low altitude ones, following Bergmann´s rule (i.e. larger body size in colder environments). This is in sharp contrast with the vast mayority of squamates which exhibit reversed Bergmann´s clines (Ashton & Feldman, 2003). One of the reasons that could explain the deviation from the reverse Bergmann's cline in these *P. guadarramae* populations is that resource limitation impair somatic growth in warmer environments (Congdon, 1989;

Dunham *et al.,* 1989). Several studies support a link between growth rates and habitat production via climate effects that may be responsible for differential body size and life histories (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin, 1985; Buckley *et al.,* 2007; Díaz *et al.*, 2011).

In our system, the driver of body size and growth differences among high and low altitude sites seems to be food availability, which differed both between sites and seasons. Environmental constraints such as food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability (Stamps & Tanaka, 1981). The number of potential prey, size-specific and mass-specific growth rates were higher at the high altitude site. In line with these results, prey availability fluctuated seasonally and was correlated with lizard growth. Size-specific, mass-specific growth rates and prey availability were maximal in late spring. Morevover, prey diversity was also higher in spring than in late summer, so we could interpret that highlands bear also a better quality of food, considering that a higher diversity of arthropods is beneficial for a generalist species (Salvador & Carretero 2014). In desert habitats, low primary productivity is primarily caused by low water availability (Noy-Meir, 1973, 1974; Webb *et al.,* 1978; Cunningham *et al.,* 1979; Hadley & Szarek, 1981) that indirectly limits secondary production (Dunham, 1981). These environmental restrictions are common in Mediterranean lowlands where drought becomes less restrictive as altitude increases due to milder temperatures and higher precipitation, which increases productivity (Nahal 1981; Iraeta et al. 2012). Thus the resource adquisition constraints imposed in the lowland site, which are more severe in summer, may be responsible for slower growth rates as it has been demostrated in other insectivorous lizard species (Dunham 1978; Ballinger and Congdon 1980). However we cannot dismiss the possible role of limitations on resource harvest rates. In *Sceloporus merriami* the thermal environment during the summer (i.e. high temperatures) restricts lizard activity to a brief period on a daily basis (Grant & Dunham 1987). We did not examined if activity patterns vary seasonaly or between sites. Nonetheless, the effects of summer drougth and higher temperatures seem more evident in the lowland site, and few lizards use to be observed (Ortega *et al.,* 2015). Lowland lizards might reduce activity to avoid dehydration (Jones et al. 1987; Lorenzon et al. 1999). Thus, the resource limitation and the harvest rate limitation hypothesis are not mutually exclusive and might shape a landscape where the lowland site is a poor environment for food acquisition and, hence, slower growth rates.It could be argued that as we did not release hatchling lizards immediately after hatching, keeping them in the lab till the end of winter, our results are biased. Although we understand this kind of concern, we think that this would have been a major drawback if we would not have found growth differences between release sites. However, after all, the growing environment determined juvenile size and growth rates even after overwintering lizards in the lab. It is also worth noting that to generalize our results to Mediterranean lizards in the Iberian Peninsula we would need more replicates of both highland and lowland sites to know if this pattern is general and widespread not just in *P. guadarramae* but also in other species with Mediterranean distribution. However, our results agree with most research on Mediterranean lizards where primary productivity is an important predictor of body size and/or growth (Aragón & Fitze, 2014; Iraeta et al., 2006). In keeping with this, *P. guadarramae* is also more abundant in the highland site (Ortega et al., 2015) supporting the general view that a higher environmental productivity is able to sustain higher lizards densities (Díaz & Carrascal, 1991; Díaz 1997).

In conclusion, the work presented here highlights how a life history perspective can enrich our understanding of Bergmann´s clines (Roff 1980, 1986) and emphasizes how geographical variation in body size can be produced by mechanisms not necessarily related to Bergmann’s rule (Palkovacs, 2003; Aragón & Fitze, 2014). Hatchlings released in the highland site grew faster and, hence, attained larger body sizes with independence of its population of origin, which was mediated by the higher food availability and quality at high altitude. Thus, this pattern of high morphological variability among populations is caused by the proximate effects of local conditions.

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Chapter III

This chapter reproduces entirely the manuscript:

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Conspicuous blue tails, dorsal morphs and escape behaviour in hatchling Iberian wall lizards (*Podarcis hispanicus*)

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Abstract: Predation has profound effects on the phenotypes of animal prey and, in lizards, the relationship between coloration and antipredatory behaviour has been studied in depth. However, studies that address the relationships between dorsal patterns and tail coloration with escape behaviour in polymorphic lizards are absent in the literature. We described dorsal morphs and measured tail coloration and escape behaviour in hatchling Iberian wall lizards, *Podarcis hispanicus,* a species with a previously undescribed female restricted dorsal polymorphism (reticulated-blotched males, and either striped or reticulated-blotched females) and juvenile tails with conspicuous blue coloration, which is probably used to divert predator attacks towards the autotomizable tail. Overall we provided evidence for the existence of sexual dimorphism in tail ultraviolet reflectance between reticulated females and males, with striped females being intermediate. We identified sex/dorsal morph, body size and tail brightness as predictors of different aspects of escape behaviour and suggested the existence of two alternative escape strategies between striped and reticulated-blotched females that may be dependent on dorsal morph differences, independently of the sex. Reticulated-blotched females, and also males (all reticulated-blotched), ran faster and spent less time paused than striped females, which might reflect an escape behaviour strategy based on endurance in striped females. In addition, lowland males displayed tail waving as a 'last resort' antipredator strategy that may be related to fatigue. We concluded that hatchling antipredatory behaviour is influenced by both dorsal pattern and tail conspicuousness.

Keywords: Antipredatory behaviour, intermittent locomotion, Lacertidae, sexual dimorphism, tail waving, polymorphism, ultraviolet reflectance

Introduction

Predation, as a mode of natural selection, is considered a major force in the evolution of animal adaptations (Lima & Dill, 1990; Lima, 1998). Predation may have profound effects on prey animals, modulating life-history, morphological and physiological traits (Endler, 1995; Ylönen *et al.*, 2006; Gordon *et al.*, 2009) and even coloration and behaviour (Losos *et al.,* 2004; Cheney, 2013). Among them, the effects of predation on coloration have been studied in depth. In many taxa, cryptic coloration reduces detection by visual predators and, thus, predatory attacks via background matching (e.g. Eterovick *et al.,* 1997; Cuadrado *et al.,* 2001; Rosenblum, 2006; Castilla *et al.*, 2007). However, predation-mediated selection might also promote the evolution of conspicuous coloration if the fitness benefits of exhibiting bright coloration are greater than the costs (Wilkinson, 2003). For example, in poison dart frogs, bright coloration acts as a warning signal (aposematism) of toxicity to potential predators, reducing lethal attacks after detection (Santos *et al.,* 2003; Siddiqi *et al.*, 2004). In other species, conspicuous colorations acts as a decoy to deflect attacks of predators to non vital areas of the body, increasing the probability of survival (Arnold, 1984; Wilkinson, 2003). For example, some lizards have autotomous tails with bright colours such as red, green or blue (Pianka & Vitt, 2003). The presence of this colour trait in phylogenetically distant species of diverse families (Cooper & Vitt, 1985; Pianka & Vitt, 2003) suggests that this may be an important antipredation mechanism among lizards (Watson *et al.*, 2012).

The adaptive value of conspicuous colourful tails in lizards has been interpreted in light of several hypotheses. Thus, tail coloration might signal subordinance to conspecifics, or unpalatability or awareness of the prey to predators (see Arnold, 1984; Cooper & Vitt, 1985 for a review). However, the most widespread and widely accepted is the `predator escape´ hypothesis which argues that conspicuous tails serve as a decoy, after detection by predators, to divert attacks away from vital areas, increasing strikes on the tail, and thus escape probability (Arnold, 1984; Cooper & Vitt, 1985; Castilla *et al.,* 1999; Watson *et al.*, 2012).

The underlying basis of drawing predator attacks towards the lizard tail is the ability to autotomize the tail of many lizards (Zani, 1996; Bateman & Fleming, 2009; Higham *et al.,* 2013). In some cases, lizard prey intentionally direct attacks by vibrating, undulating or waving movements of the tail (Congdon *et al.,* 1974; Mori, 1990; Cooper, 1998, 2001; Telemeco *et al.,* 2011). Thus, the brightly tail coloration may enhance the already effective antipredatory autotomy mechanism (Cooper & Vitt, 1985**,** 1991). Nevertheless, tail displays might also be a pursuit-deterrence signal that warns predators that they have been detected, prompting predators to abort attacks (Dial, 1986; Hasson *et al.,* 1989; Cooper, 2007).

The Iberian wall lizard, *Podarcis hispanicus* is an insectivorous and small diurnal lacertid lizard (Pérez-Mellado, 1998). It has a wide distribution througthout the Iberian Peninsula and North-West Africa (Guillaume, 1987). Juveniles have a distinct bluish-green tail coloration (Castilla *et al.*, 1999) (see Fig. 1). In the closely related species *P. bocagei*, hatchlings are known to do undulating or waving movements of their green tails to human observers (Galán, 1994). In addition, although overlooked in the scientific literature, *P. hispanicus* shows a dorsal pattern polymorphism in males and females (striped vs. reticulated-blotched; see results and Fig. 2) in many population and lineages like those object of this work. Contrasted differences in predation risk between sexes might lead to intersexual differences in antipredatory behaviour (Shine *et al.*, 2000; Vanhooydonck *et al.,* 2007; Bohórquez Alonso *et al.*, 2009). However, many studies have demonstrated that colour patterns and behaviours may be selectively and genetically coupled in polymorphic species (Brodie, 1989; King, 1993; Forsman & Shine, 1995). Therefore, this morph variation might also affect antipredatory behaviour. In fact, covariation between spotted and striped dorsal patterns with escape behaviour has been demonstrated in adults of the Moroccan rock lizard, *Scelarcis perspicillata* (Carretero *et al.*, 2006). Despite these evidences, studies that characterise intersexual and intermorph spectral tail coloration variability, or that experimentally address the relationships between dorsal and tail coloration with escape behaviour, including tail waving are absent in the literature.

Figure 1. Picture of a reticulated-blotched P. hispanicus female showing the conspicuous blue-green tail coloration.

Here, we described characteristics of dorsal morphs and measured coloration spectral properties of tails of hatchling *P. hispanicus*. We also conducted an experiment in the laboratory to characterise escape behaviour and tested whether tail coloration and dorsal morph were predictive of escape behaviour. Specifically, our *P. hispanicus* populations show a female-limited pattern polymorphism with two alternative dorsal patterns in females, striped and reticulated-blotched. Thus, we hypothesised that tail conspicuousness, as a decoy according to the `predator escape´ hypothesis; and escape behaviour would be coupled with dorsal morphs, independently of sex, as males share the reticulated-blotched pattern with one female category. We predicted that striped females would have more conspicuous tails and would escape at higher sprint speeds than reticulated-blotched hatchlings (both males and females), as expected from an escape strategy based on flight. In contrast, we expected that reticulated-blotched hatchlings would have duller tails and would escape by running slower and with more pauses, in accordance with an antipredatory strategy based on crypsis. Most studies show that visual conspicuousness of lizards correlates positively with shyness, (Hedrick, 2000; Cuadrado *et al.*, 2001; López *et al.*, 2005 Lindström *et al.*, 2007; Cabido *et al.*, 2009). Hence, we predicted that hatchlings with brighter tails, due to its higher conspicuousness to predators, would show a more wary behaviour, reducing the number of pauses during escape episodes as a mode of behavioural compensation. Furthermore, preliminary field observations have noted that, when pursued by man, juvenile *P. hispanicus* do conspicuous undulating movements of the tail after repeated chases (J. Ortega pers. observ.). We hypothesized that this tail waving should function as a 'last resort' strategy in response to fatigue rather than a pursuit deterrence signal.

In addition, population differences in predation pressure have been suggested as the cause of population differences in coloration (Baird *et al.,* 1997; Macedonia *et al.,* 2002; Stuart-Fox *et al.*, 2004) including variations in the occurrence of bright tail coloration (Brown & Thorpe, 1991). We studied here lizards from two close populations located along an altitudinal gradient. Thus, we hypothesized that hatchlings from the different populations might differ in tail conspicuousness and escape behaviour in response to potential altitudinal differences in predation pressure. Because lizards from highland populations are expected to suffer lower predation intensity (Van Damme *et al.*, 1989; Fox *et al.,* 1994), we predicted that lowland hatchlings would have more conspicuous tails and would display tail waving more frequently than highland hatchlings.

Material and methods

Study animals

Gravid female *P. hispanicus* were captured by noosing at two nearby populations in the Guadarrama Mountains (Central Spain) in April-May 2011. These population 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 (40º44´N, 04º02´W; 1,250 m altitude). The highland locality is found in the upper part of `Fuenfría´ Valley (40º47´N, 04º03´W; 1,750 m altitude) (for details of environmental characteristics of these populations and morphological differences between adult lizards see Gabirot *et al.,* (2013). It is worth noting that the *P. hispanicus* populations within this geographical area have just been named as a separated species within this complex (*P. g. guadarramae*; see Geniez *et al.*, 2014).

Females from each population were kept separately at "El Ventorrillo" field station facilities in two different climatic walk-in chambers (Ibercex V-450-D; ASL S.A., Madrid, Spain) where temperature (diurnal = 21ºC; nocturnal = 15ºC) and photoperiod (12 h:12 h, light:dark) were easily controlled automatically. Lizards were kept in individual plastic terraria (40x30x25 cm) until oviposition. We provided a moistened substrate, a brick (24x11x8 cm) for shelter and climbing opportunities, ultraviolet radiation during 1.5 h a day and a hot spot for thermoregulation that allow lizards to attain body temperatures within their preferred temperature range (34.4 °C; Bauwens *et al.*, 1995). Crickets (*Acheta domesticus*) and mealworms (*Tenebrio molitor*) as food were provided *ad libitum* and water with excess. In the laboratory, 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 ºC (IRE-160; 94x60x60 cm; Raypa, Barcelona). Eggs were randomly distributed in the incubator and the shelves rotated each week to control for possible position effects (Telemeco *et al.*, 2010).

We examined daily the incubator for hatching. Hatchlings lizards were kept in individual plastic terraria in the same conditions detailed above for adults, but they were fed small prey: pinhead crickets (*Acheta domesticus*), fruit flies (*Drosophila hydei* and *D. melanogaster*) and small waxworms (*Galleria mellonella*). Lizards were released at the end of the experiments in their capture places, or in those of their mothers.

We measured newborn lizards prior to escape trials. Body size was measured with a rule (snout-vent length, 'SVL'; to the nearest 1 mm) and 'body mass' using a digital scale (to the nearest 0.01 g). 'Femoral length' was measured with a digital caliper (to the nearest 0.05 mm) as the mean distance from the hip to the knee of both hindlimbs. 'Body condition' of hatchlings was estimated using the residuals of the regression of log-body mass on log-SVL at hatching. Hatching date was referred to May 1st, and, thus, transformed into a continuous variable like Julian days (Warner & Shine, 2009). Sex was assessed, and confirmed several times during the experiment, by the highly developed femoral pores in males compared to females.

Tail coloration measurements

Tail coloration was measured in 50 unrelated hatchling lizards a few days after they were born (16 ± 1.7 days of age). Due to logistic problems we were not able to measure hatchlings of exactly the same age, but this was later corrected statistically. We used an Ocean Optics USB 2000 spectrophotometer to measure reflectance using a dual DT-1000-MINI Deuterium–Halogen light source (Ocean Optics, Inc., Dunedin, FL, USA). A custom-made probe holder oriented at 45º and 1 cm away from the skin surface was used to exclude ambient light and standardise measuring distance. Each spectral reading consisted of percent reflectance recordings in reference to a white standard. Blue and green coloration in reptiles is a structural color produced by the interaction of dermal chromatophores, specifically iridiophores underlined by melanophores (Rohrlich, 1974). This melanin layer functions as an absorber of wave-lengths other than blue (Bagnara *et al.,* 2007). As melanin based coloration is known to be affected by temperature and other stressful factors (Norris, 1965; Filadelfi & Castrucci, 1994), spectrophotometric recordings were taken after allowing lizards to termorregulate for 1 h in their terraria. We took two measurements, one near the base and another at the tip of the tail, restricting measurements to the "blue" (for the human-eye) area of the tail. Because we did not find any significant differences between tail regions in preliminary analyses ($P > 0.732$ in all cases, when comparing PC scores from a principal components analysis including all measurements) we averaged the tail measurements for each individual to be used in further analysis.

Spectral raw data were processed with CLR 1.1. software (Montgomerie, 2009) and reflectance readings from 300 to 700 nm, summarized over 5 nm steps ("binned"; Grill & Rush, 2000), were selected for analysis, as they represent the spectral range visible to squamates and their avian predators (Bennett & Cuthill, 1994; Cuthill *et al.*, 1999). A principal components analysis (PCA) was performed with coloration measurements. As a result, three main components were obtained (tail-PCs; see results). The PCA summarises all of the information about the shape of complex reflectance spectra, including bimodal ones, such as those found here (see Fig. 4), into a few PCs independent from each other. In PCA of spectral data, PC1 represents variation in intensity of coloration or brightness, and subsequent PCs represent combinations of hue and chroma (Endler, 1990; Cuthill *et al.*, 1999; Grill & Rush, 2000). Also, the PCA identifies those sections of the spectrum (wavelength regions) that are contributing to the observed variation, independently of their “importance” in terms of contribution to the total amount of reflectance (Montgomerie, 2009).

To compare coloration, we summarised sex and dorsal polymorphism in a single variable (‘sex/dorsal morph’) with three levels: reticulated-blotched female, striped female and male (males are monomorphic with a reticulated-blotched pattern; see results). We used General Lineal Models (GLMs) with each of the tail-PC scores describing coloration as dependent variables and with ‘sex/dorsal morph’ as a fixed factor and ‘population’ as a random factor. We also included in the initial models hatchling ‘date of birth’ and ‘SVL’ as covariates to correct for the potential effects of age and size of lizards when they were measured.

Escape behaviour

Locomotor escape behaviour and tail waving behaviour were recorded in the same 50 hatchling lizards for which, two days before, we had measured tail coloration. Before the trials, we standardised body temperature of lizards by placing hatchlings in an incubator at 35.5 °C for 30 min, which is the optimal temperature for sprint performance in *P. hispanicus* (Bauwens *et al.*, 1995). We simulated a predator attacking from behind by tapping lizards close to the tail with a brush to stimulate them to run along a 1 m treadmill with a cork substrate that provided excellent traction. Cenital filming was performed with a miniDV video camera (JVC GR-DVL365EG, Japan) recording at constant 25 frames s^{-1}. Each lizard was run four consecutive times with at least a 10 min resting period between successive trials to avoid stress and fatigue (Braña & Ji, 2000). Videotapes were examined frame by frame, and we noted distance and time moved by lizards using the tip of the snout as a reference (Martín & Avery, 1998). ‘Sprint length’ was considered as the total distance ran before stopping for 2 s. ‘Total sprint time’ was the time spent running the total sprint length. ‘Mean speed’ was calculated over the total sprint length. The fastest four consecutive frames interval over the four trials was considered to be the ‘maximal sprint speed’. ‘Number of pauses’ (shorter than 1 s) and ‘time spent in pauses’ during the trials were also recorded. We also measured the ‘number of tail waving episodes’ and the ‘tail waving time’ per episode. Except for ‘maximal sprint speed’, all variables were averaged for the four trials of each individual and means were included in subsequent analyses (Table 1).

To summarise escape behaviour, we performed a PCA on the eight variables that described escape (see Table 1). We used the PC scores of individuals from the three main components obtained (escape-PCs; see results) in subsequent analyses. We used General Lineal Models (GLMs) to seek out for differences in escape-PC scores describing escape behaviour among ‘sex/dorsal morph’ (fixed factor) and between‘populations’ (random factor), and included the interactions between these two factors in the models. In the initial models, we also included ‘SVL’ and ‘date of birth’ as covariates to remove the possible effect of these factors on the main results. Post hoc pairwise comparisons were planned using Tukey’s honestly significant difference tests (Sokal & Rohlf 1995). We also used a generalized linear model (GLZ) for a binomial variable with a logit link function to examine the effects on frequency of tail waving behaviour of population, sex/dorsal morph and sprint speed. For the latter, we categorized individual lizards as ‘fast’ or ‘slow’, by sorting those individuals whose maximal sprint speeds were above than the entire group median as ‘fast’, and those individuals whose maximal sprint speeds were below than the group median as ‘slow’.

To determine how tail coloration influenced escape behaviour, all three tail coloration principal components (tail-PC´s) together with ‘sex/dorsal morph’, ‘SVL’, ‘tail length’, ‘femoral length’, ‘body mass’ and hatchling’s ‘date of birth’ (independent variables) were initially included in three backward stepwise multiple regression models, one for each of the three escape-PCs describing escape behaviour as dependent variables. We presented here final models that retained only significant variables. We detected two outliers (one male and one female) within our dataset and, thus, we performed the backward stepwise multiple regression models without these two cases.

Results

Dorsal morph description

In our study populations, two clearly distinguishable dorsal patterns occur (Fig. 2). A 'striped' pattern appears only in females (Fig. 2B); it has two whitish dorsolateral stripes, each one surrounded by two black stripes that run from the base of the head and reach the base of the tail. The dorsal black stripes are wider and delimitate a brown mid-dorsal band. The dorsolateral black stripes are much thinner, with irregular edges, sometimes discontinuous and, in a few cases, they are absent. The second morph, 'reticulated-blotched', is more variable and appears in all males (Fig. 2C) and in some females (Fig. 2A). Indeed, the ranges of inter-individual variation overlap between sexes. The dorsolateral whitish stripe is replaced by whitish dots. These dots are surrounded by either black blotches, which are more abundant in the dorsal area, or a black reticulated matrix. Sometimes both the black and the whitish patches of coloration can be quite close to each other and aligned with the body sagital axis, giving a slightly striped appearance. Thus, dorsal pattern polymorphism is female restricted in our study populations. Adult striped females were significantly more abundant than reticulated-blotched females (63 % vs. 37 % respectively; $\chi^2 = 4.625$, $P = 0.031$) and there were not significant differences in frequencies between populations ($\chi^2 = 0.551$, $P = 0.475$). Given this overview, we classified our hatchlings in three different categories that incorporated both sex and dorsal polymorphism: striped females, reticulated-blotched females, and reticulated-blotched males.

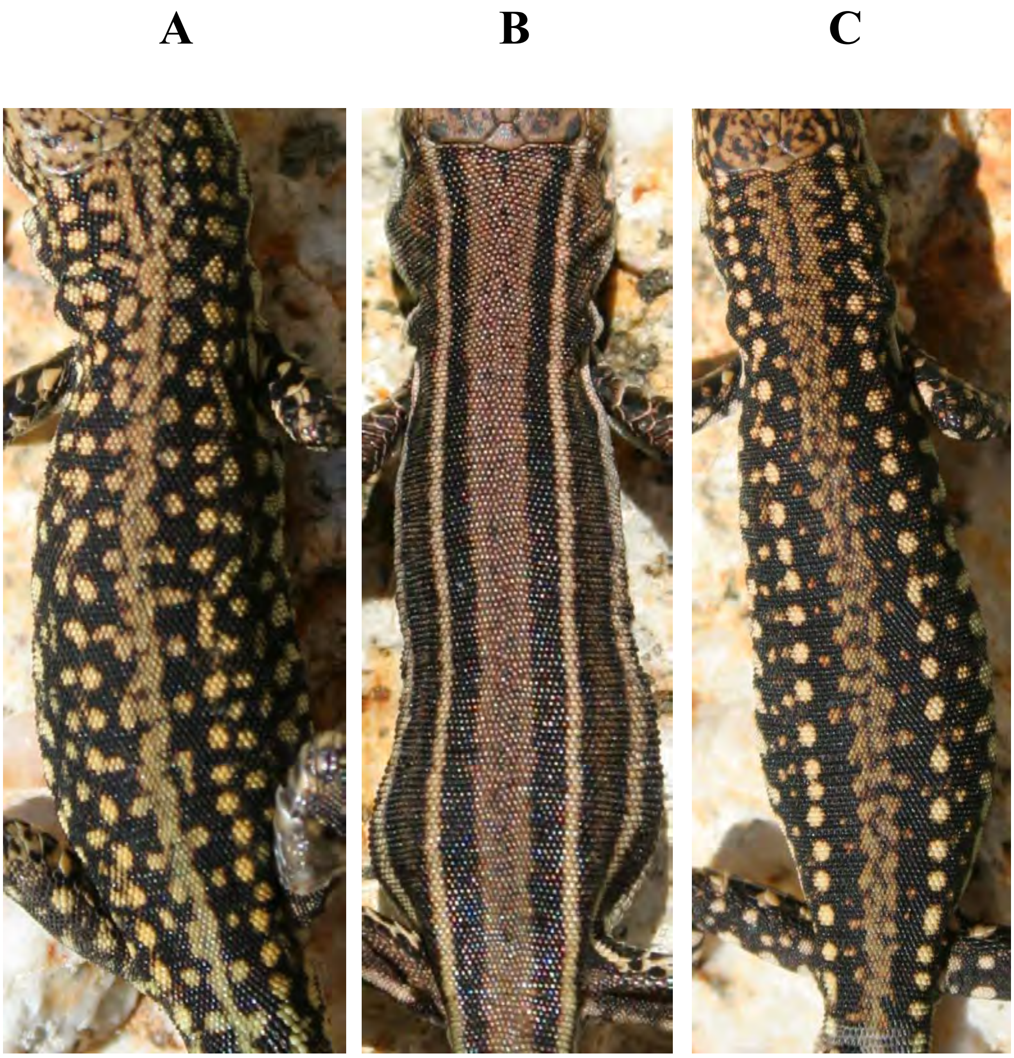

Figure 2. Photographs showing dorsal pattern polymorphism in our P. hispanicus populations. A, reticulated-blotched female; B, striped female; C, reticulated-blotched male.

Morphological biometrical variables did not differ between populations (GLMs for femoral length, body condition and SVL with hatchling date of birth as a covariant and population and sex/dorsal/morph as random and fixed factors, respectively; all P´s > 0.422). The only morphological biometrical variable that differed between sex/dorsal morph of hatchling lizards was tail length (reticulated-blotched females: 43 ± 1 mm; striped females: 38 ± 1 mm; reticulated-blotched males: 40 ± 1 mm) (GLM; hatchling date of birth: $F_{1,46} = 3.91$, $P = 0.054$; sex/dorsal morph: $F_{2,46} = 3.44$, $P = 0.040$). Tukey´s post hoc tests revealed significant differences between reticulated-blotched and striped females (*P* = 0.035), while males did not significantly differ of reticulated blotched (*P* = 0.191) or striped females (*P* = 0.531). The remaining morphological variables were not significantly different between sex/dorsal morphs (GLMs for femoral length, body condition and SVL with hatchling date of birth as a covariant; all *P*′s > 0.146).

Tail coloration

The PCA on reflectance data of all spectra of tail coloration produced three principal components (tail-PCs) that together accounted for 98.39 % of the variation in the original spectra (Fig. 3). The first PC (tail-PC1) accounted for 82.71 % of variation (eigenvalue = 66.99). Coefficients relating tail-PC1 to the original reflectance data were all negative and of similar magnitude, so tail-PC1 represented achromatic brightness variation in the original spectra (Fig. 3). The second PC (tail-PC2) accounted for a further 9.92 % of the variation (eigenvalue = 8.04) in the original spectra and the pattern of coefficients suggested that positive values represented variation in both short and medium (410-645 nm) wavelengths while negative values reflect variation in both very short (300–405 nm) and very long (650–700 nm) wavelengths. Thus tail-PC2 represented variation in the relative amount of short, UV to long, visible, wavelength reflectance, with more negative tail-PC2 scores indicating more ultraviolet reflectance (< 400 nm). The third PC (tail-PC3) accounted for 5.76 % of the variation (eigenvalue = 4.66). The coefficients relating thPC3 to the original reflectance values below 555 nm were all positive, while above 555 nm they were negative. Thus, more positive tail-PC3 scores indicated more saturated bluish tail colours, and negative values indicated more brownish tail colours (i.e. more similar to those of adults' tails).

Initial GLM models showed that the effects of population and date of birth described by the three tail-PCs were not significant in any case (population: all P′s > 0.216; date of birth all P′s > 0.657). Thus, because tail coloration did not vary between populations or depending of the age of the lizards, we excluded these factors and retained sex/dorsal morph as the only factor for subsequent analyses. Brightness (tail-PC1) did not significantly differ between sex/dorsal morphs (GLM; sex/dorsal morph: $F_{2,47}= 0.56$, $P = 0.574$). However, UV coloration (tail-PC2) showed significant differences between sex/dorsal morphs (GLM; sex/dorsal morph: $F_{2,47}= 4.01$, $P = 0.025$). Both striped and reticulated-blotched females had higher UV reflectance than males. Nevertheless, Tukey post hoc tests showed that the only significant difference occurred between reticulated-blotched females and males ($P = 0.031$), whereas the rest of comparisons did not reach significance ($P > 0.130$ in all cases) (Fig. 4, 5). Finally, with respect to bluish coloration (tail-PC3), we did not find any significant difference among sex/dorsal morphs (GLM; sex/dorsal morph: $F_{2,47} = 2.13$, $P = 0.131$ (Fig. 4, 5).

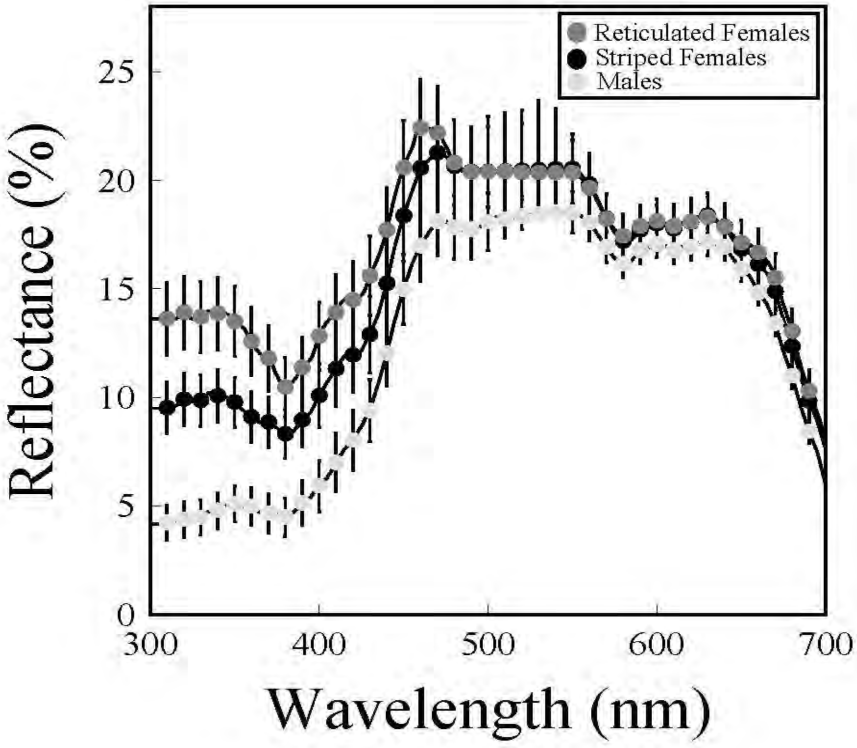

Figure 3. Coefficients of the first three principal components from a PCAs on reflectance spectra that characterize tail coloration in hatchling P. hispanicus.

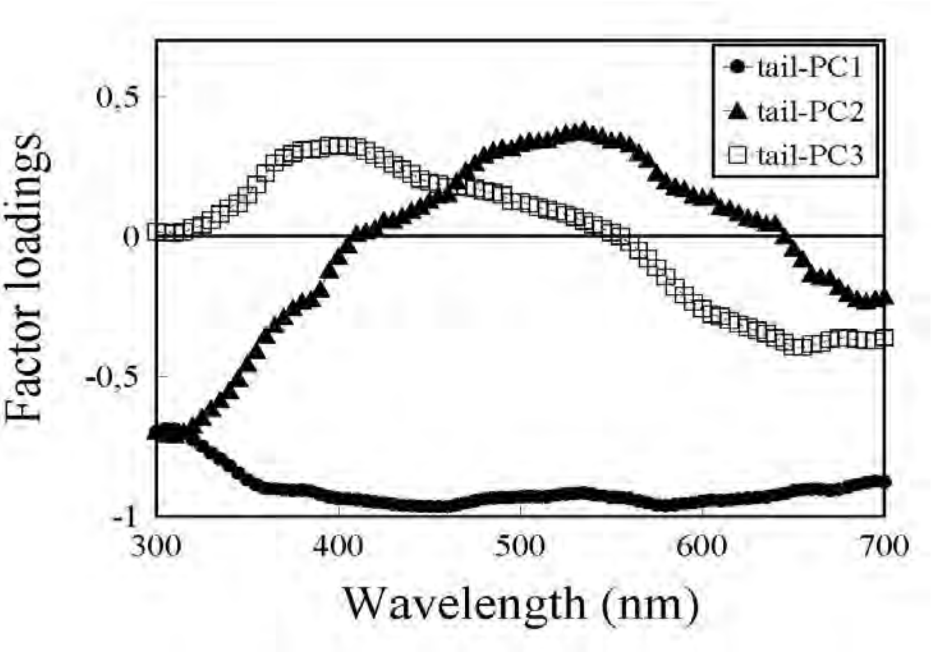

Figure 4. Mean (±SE showed at 5-nm intervals) reflectance spectra of the tail coloration in the three sex/dorsal morph categories in hatchling P. hispanicus.

Escape behaviour

The eight escape behaviour variables (Table 1) were summarised using a PCA which produced three main components that together accounted for 78.79 % of the variation. The first one (escape-PC-1) explained 41.32 % of variance (eigenvalue = 3.31) and was positively correlated with mean and maximal sprint speed and negatively correlated with time spent in pauses and total sprint time. The second main component (escape-PC2) explained 24.70% of variance (eigenvalue = 1.98) and was positively correlated with sprint length and negatively correlated with the number of tail waving episodes and tail waving time. The third one (escape-PC3) explained 12.78 % of variance (eigenvalue = 1.02) and was negatively correlated with number of pauses.

Initial GLM models showed that the effects of date of birth and SVL on escape behaviour described by the three tail-PCs were not significant in any case (date of birth: all

*P′*s > 0.151; SVL: all *P′*s > 0.119). Thus, because escape behaviour did not depend on the age and size of the lizards, we excluded these factors and retained population and sex/dorsal morph for subsequent analyses.

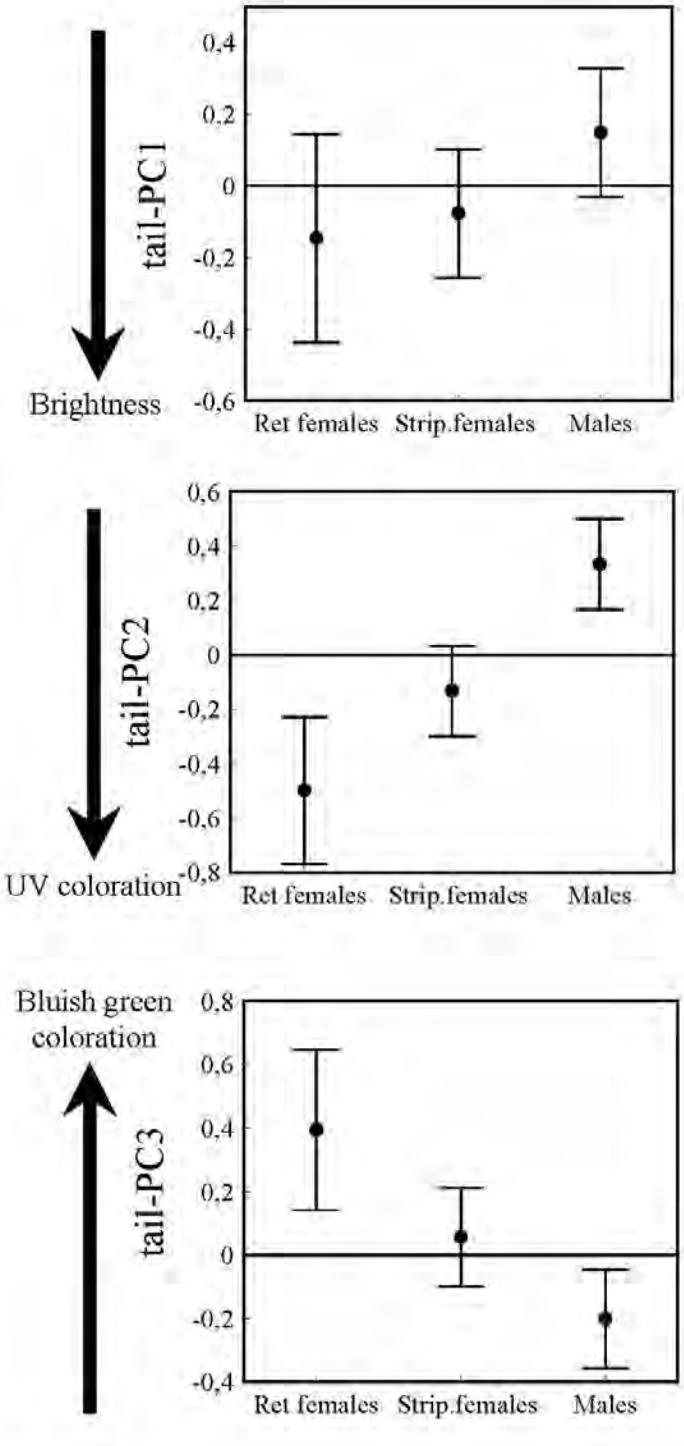

Figure 5. Mean (± 1 SE) values of tail-PCscores describing brigthness (tail-PC1), ultraviolet (tail-PC2) and blue-green (tail-PC3) of tail coloration in the three sex/dorsal morph categories in hatchling P. hispanicus.

We did not find significant differences between populations but there were significant differences among sex/dorsal morph in escape-PC1 scores (GLM; population: $F_{1,44} = 0.84$, $P = 0.394$; sex/dorsal morph: $F_{2,44}= 26.33$, $P = 0.037$; population x sex/dorsal morph interaction: $F_{2,44} = 0.16$, $P = 0.853$; see average values in Table 1; Fig. 6). Due to the lack of interpopulational differences, a further GLM excluding population was performed to improve the significance of the model (GLM; sex/dorsal morph: $F_{2,47} = 5.05$, $P = 0.010$). Post hoc Tukey´s comparisons showed that striped females spent significantly more time in pauses per race and had significantly lower mean and maximal sprint speed than males ($P = 0.008$), while reticulated-blotched females were intermediate and did not significantly differ of males ($P= 0.579$) or striped females ($P = 0.374$).

With respect to the escape-PC2 scores, there were not overall significant differences among sex/dorsal morph or between populations, but the interaction was significant (GLM; population: $F_{1,44} = 0.42$, $P = 0.583$; sex/dorsal morph: $F_{2,44} = 0.67$, $P = 0.599$; population x sex/dorsal morph interaction: $F_{2,44} = 9.57$ $P < 0.001$) (Fig. 6). Separate GLM for each sex/dorsal morph suggested that lowland males from Golondrina tail waved more than highland ones from Fuenfría (GLM: reticulated-blotched males: $F_{1,19} = 13.06$, $P = 0.002$; tail waving frequency, mean ± SE; Fuenfría: 0 ± 0.08, Golondrina: 0.70 ± 0.11), whereas there were not significant differences between populations of females of the two morphs (GLM´s; reticulated-blotched: $F_{1,6} = 0.14$, $P = 0.718$; striped: $F_{1,19} = 1.95$, $P = 0.179$).

Finally, we did not find significant differences between populations or among sex/dorsal morphs for number of pauses while escaping (i.e. escape-PC3 scores) (GLM; population: $F_{1,44} = 0.55$, $P = 0.494$; sex/dorsal morph: $F_{2,44} = 1.29$, $P = 0.437$; population x sex/dorsal morph interaction: $F_{2,44} = 0.24$, $P = 0.787$) (Fig. 6).

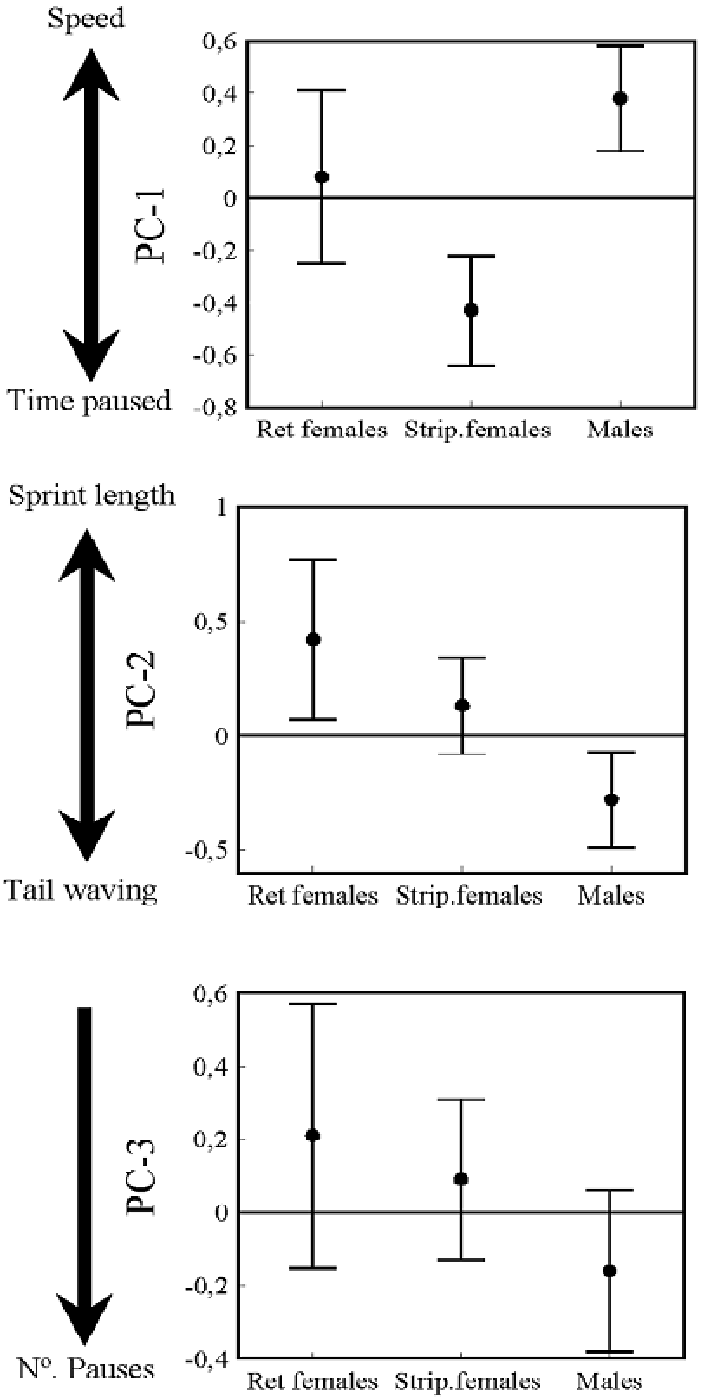

Figure 6. Mean (± 1 SE) values of escape-PC scores describing escape behaviour of the three sex/dorsal morph categories in hatchling P. hispanicus.

12% of hatchlings (6 of 50) tail-waved and 100 % (6 from 6) of the hatchlings that tail waved, did it in the last section of the treadmill where the wall would impede further running.

Hatchlings from Golondrina were significantly more prone to tail wave than those from Fuenfría (GLZ; $\chi^2_1 = 4.56$, $P = 0.033$; tail waving frequency, mean ± SE; Fuenfría: 0.03 ± 0.03, Golondrina: 0.25 ± 1.00). However we did not find a significant effect of sex/dorsal morph ($\chi^2_2 = 1.98$, $P = 0.372$) or hatchling speed ($\chi^2_1 = 1.52$, $P = 0.217$). As none of the interactive effects were significant (all *P′*s > 0.99), we removed them from the final GLZ model.

Table 1. Mean (± 1 SE) values for the escape variables recorded during the experiments in the three sex/dorsal morph categories in hatchling P. hispanicus. Number of individuals in parentheses.

	Reticulated-blotched females (*n* = 8)	Striped females (*n* = 21)	Males (*n* = 22)
Total sprint time (s)	1.44 ± 0.11	1.42 ± 0.07	1.24 ± 0.07
Sprint length (cm)	67.3 ± 3.0	61.2 ± 1.8	61.4 ± 1.8
Time spent in pauses (s)	0.26 ± 0.07	0.31 ± 0.04	0.17 ± 0.04
Number of pauses (n)	1.8 ± 0.5	2.0 ± 0.3	2.1 ± 0.3
Maximal sprint speed ($cm \cdot s^{-1}$)	89.6 ± 6.5	83.3 ± 4.0	92.6 ± 3.9
Mean speed ($cm \cdot s^{-1}$)	50.2 ± 3.3	43.9 ± 2.1	52.4 ± 2.0
Number of tail waving episodes (n)	0.04 ± 0.13	0.03 ± 0.08	0.22 ± 0.08
Tail waving time (s)	0.01 ± 0.04	0.01 ± 0.02	0.07 ± 0.02

Relationship between escape behaviour and tail coloration

Backward stepwise multiple regression models showed that escape-PC1 was only significantly influenced by hatchling sex/dorsal morph in the final model (Model: $R^2 = 0.18$, $F_{2,45} = 4.98$, $P = 0.011$), independently of their morphological and tail coloration characteristics. Post hoc Tukey´s comparisons showed that striped females spent significantly more time in pauses per race and had significantly lower mean and maximal sprint speed than males ($P = 0.008$), while reticulated-blotched females were intermediate and did not significantly differ of males ($P= 0.596$) or striped females ($P = 0.348$). Second, escape-PC2 was significantly influenced only by body size (Model: $R^2 = 0.12$, $F_{1,46} = 6.46$, $P = 0.014$; SVL: $\beta = 0.35 \pm 0.14$, $t = 2.54$, $P = 0.014$). Thus, larger lizards ran significantly longer distances and displayed significantly less tail waving episodes, independently of their sex/dorsal morph and other morphological and tail coloration variables. Finally, escape-PC3 was significantly influenced by tail brightness (tail-PC1) (Model: $R^2 = 0.12$, $F_{1,46} = 6.46$, $P = 0.014$; tail-PC1: $\beta = -0.30 \pm 0.14$, $t = -2.12$, $P = 0.039$). Thus, the brighter the tail, the less number of pauses that lizards did, independently of their sex/dorsal morph and other variables.

Discussion

Overall, our results do not support a relationship between dorsal pattern and tail conspicuous coloration. We found that neither sex nor dorsal morphs of hatchling *P. hispanicus* differed in the visible spectra composition of tails. However, hatchling reticulated females have more ultraviolet (UV) reflectance in their tails than striped females and reticulated-blotched males, while striped females have intermediate UV reflectance and males the lowest UV reflectance.

Considering that these conspicuous tails very likely are useful to divert predator attacks towards the autotomizable tail (Watson *et al.*, 2012), differences in tail conspicuousness in the UV range may be associated with differential predation risk between sex/dorsal morphs and/or the use of different antipredatory strategies. Although sexual differences in antipredatory strategies are present in several species of reptiles (Shine *et al.* 2000; Plasman *et al.*, 2007; Vanhooydonck *et al.*, 2007) this is the first study that describes sexual dimorphism in tail conspicuousness in hatchling lizards. Several alternative explanations that invoke predation as the major selective force could account for UV reflectance differences, such as differential microhabitat selection or foraging behaviour. Indeed, active foraging behaviour and selection of open microhabitat have been linked to more conspicuous tails in *Acanthodactylus beershebensis* lizards, where blue tailed hatchlings forage actively compared to adults and spend a larger time in open habitats than dull tailed ones (Hawlena *et al.*, 2006). Also, physiological differences between sexes or trade-offs between other behavioural traits could constraint the evolution of high UV reflectant tails in males. Nonetheless, the relative contribution of these factors is not addressed in the present work and should be examined in further studies that tease apart such possibilities. Also, we did not examine other antipredatory strategies which might differ between sexes and morphs, and might explain differences in UV reflectance.

The differences in UV reflectance between reticulated-blotched hatchlings, both males and females, is in sharp contrast with their escape behaviour similarities as they showed higher mean and maximal speed, and spent less time in pauses than striped females. This suggests an association between dorsal morph and some aspects of escape behaviour independently of the sex. Similarly, in two syntopic forms differing in dorsal pattern of the Moroccan rock lizard (*Scelarcis perspicillata*), spotted individuals have longer flight initiation distances and take more time to abandon shelter because they are shier than striped lizards (Carretero *et al.*, 2006). However, Calsbeek & Cox (2012) did not find a relationship between dorsal pattern and escape behaviour in *Anolis sagrei*, a species with a female-limited pattern polymorphism. However, these authors measured several escape behaviour variables, such as sprint speed, endurance and responses to an approaching investigator (freeze vs run) (Calsbeek & Cox, 2012), but not intermittent locomotion. Although we have not assessed endurance, we might interpret the escape behaviour of striped female *P. hispanicus* as one based on endurance rather than on sprint speed, which, in contrast, would be the preferred tactic of reticulated-blotched hatchlings. Striped females spent more time paused, which could enhance recovery and strengthen endurance. On the other hand, the lower maximal and mean speed in striped females could allow them to escape for longer times. Thus, endurance while fleeing from a predator could be of great importance in spite of their lower speed. In contrast, hatchling males, together with reticulated-blotched females, had faster mean and maximal sprint speeds and made shorter sprints. Thus we can infer that their escape strategy would not depend on endurance but rather on short flight bursts at the fastest as possible high speed. Thus, short sprints at high speed coupled with a more cryptic dorsal pattern might be selected in males and reticulated-blotched females. Further research should aim to measure potential variation in other aspects of escape behaviour, such as endurance or flight initiation distance, between sexes and dorsal morphs; and even extend these studies to adults, which maintain the dorsal polymorphism (J. Ortega pers. observ.).

We found that the only tail coloration trait affecting escape behaviour was tail brightness, independently of sex or dorsal morph. Differences in UV reflectance did not seem to affect differences in escape behaviour. This is not a drawback of our results as UV reflectance might affect other aspects such as microhabitat use, which are not studied here. Most studies show that visual conspicuousness of lizards correlates positively with shyness, particularly in males (Hedrick, 2000; Cuadrado *et al.*, 2001; Lindström *et al.*, 2007; López *et* *al.*, 2005). Cabido *et al.* (2009) found that male rock lizards *Iberolacerta monticola* with more shoulder ocelli, which reflect UV radiation, behaved shyer, very likely to compensate for their increased conspicuousness. Our results agree with this hypothesis, hatchlings with brighter tails did less pauses between sprint bursts than those with duller tails, adopting a shier behaviour that may minimise predation risk during flight episodes. On the other hand, hatchlings with duller tails may benefit from pauses during locomotion. Lizard intermittent locomotion occurs in many behavioural contexts including general locomotion, search and pursuit of prey, and escape from predators (Avery *et al.*, 1987). Several energetic and perceptual benefits may arise when individuals move in a discontinuous way (Kramer & McLaughlin, 2001). Among them, stimulus detection and predator avoidance might explain why hatchling *P. hispanicus* alternate short periods of locomotor activity with short pauses. There is some evidence that speed reduces perception of moving lizards (Avery, 1993) and short pauses during locomotion increase detection of both predators and prey (O'Brien *et al.*, 1990; Trouilloud *et al.*, 2004; López & Martín, 2013). Thus, brief stops during escape could improve predator vigilance and microhabitat perception, and hence, allow hatchlings to adjust their speed or change their sprint direction. Pauses may also cause predators to lose contact with an already detected prey (Edmunds, 1974; Herzog & Burghardt, 1974) via background matching (Martín & López, 1995, 1996) or by reducing the ability of predators to anticipate future prey position (Driver & Humphries, 1988). Hence, hatchlings with brighter tails might not benefit from background matching during pauses because their conspicuous tails make them more detectable than duller tailed hatchlings. Thus, we can consider two alternative antipredator strategies with relationship to the tail characteristics: hatchlings with relatively duller tails that rely on pauses to confuse predators, and hatchlings with brighter tails that show behavioural compensation, reducing the number of pauses due to their higher detectability.

Despite divergent adult morphology and coloration between Fuenfría and Golondrina populations (Gabirot *et al.*, 2013), we did not detect any inter-population differences in tail brightness, UV or bluish coloration of hatchling lizards. Contrasting selective forces between populations have been proposed as the cause of geographic variation in the occurrence of bright tail coloration (Brown & Thorpe, 1991) and reduced predation in high elevation localities has been acknowledged in some studies (Van Damme *et al.*, 1989; Fox *et al.*, 1994). However, even if these differences occurred in our study populations they do not seem to affect tail coloration of hatchling lizards. We did not describe differences in predator communities composition or in relative abundance, thus depending on the sensory biases of dominant predators in each locality we would expect different antipredatory prey phenotypes. Indeed, our results show that lowland hatchlings tail wave more frequently than highland ones. A positive association between prey response and the intensity of threat that they perceive has been widely documented (Stankowich & Blumstein, 2005).Thus, lowland Golondrina males might suffer a higher predation pressure than their highland counterparts as they were more prone to tail wave. In addition, tail waving is only displayed in the last section of the racetrack and sprint speed has no effect in tail waving behaviour. If tail waving was a pursuit-deterrent signal, faster individuals should display this behaviour more frequently than slower animals as they have the highest probabilities of escaping predators (Telemeco *et al.*, 2011). Furthermore, body size influenced sprint length and tail waving behaviour in *P. hispanicus*, with larger hatchlings running longer distances and tail waving less than smaller hatchlings. In this context, hatchlings might flee until their muscles fatigue, at which point they would be forced to implement an alternative antipredator tactic such as tail waving (Telemeco *et al.*, 2011). These findings support our prediction that tail waving occurs as a ‘last resort’ strategy that may be related to fatigue in *P. hispanicus* as it is documented in the skink *Bassiana duperreyi* (Telemeco *et al.*, 2011).

Overall, we provided evidence for the existence of sexual dimorphism in tail UV reflectance of *P. hispanicus* hatchlings, which is more pronounced between reticulated-blotched females and males, with striped females being intermediate. In addition, we identified sex/dorsal morph, body size and brightness as predictors of different aspects of escape behaviour, and two alternative escape strategies between striped and reticulated-blotched hatchlings that are probably dependent on dorsal morph differences, independently of sex. We concluded that hatchling antipredatory behaviour is influenced by the interactions between dorsal patterns, size and tail conspicuousness.

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Chapter IV

This chapter reproduces entirely the manuscript:

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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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Abstract: 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 pleitropy 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.

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 & 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, 2011; Ortega *et al.,* 2014) a bias towards insect studies exists, whereas vertebrates have been less studied (Paemelaere *et al.,* 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 *et al.,* 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 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 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 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 to carotenoid-based traits (Stoehr, 2006; Galván & Alonso-Alvarez, 2008; Metcalfe & Alonso-Alvarez, 2010). However, this relationship has rarely been explored in lizards. Vroonen et al., (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 & Oris, 1995; Arendt, 1997; Arendt, 2003). Hence, in accordance with the fast growers sprint lower hypothesis (Álvarez & Metcalfe, 2007; Shaun et al.,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 *et al.,* 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 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 *et al.,* (2004) examined the degree to which differences in reproductive allocation reduce burst swimming performance in the Trinidanian 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 (40º44´N, 04º02´W; 1,250 m altitude). The highland population is located at the upper part of `Fuenfría´ Valley (40º47´N, 04º03´W; 1,750 m altitude). For details on interpopulational differences in environmental characteristics and morphology of adult lizards see Gabirot *et al.,* (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 = 21ºC; nocturnal = 15ºC) 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 ºC) 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 *et al.,* (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 *et al.,* 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 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 *et al.,* 2001; Belliure *et al.,* 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 *et al.,* 1997; Roitt *et al.,* 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; Ilmonen *et al.,* 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 $V=(4/3)\pi\alpha b^2$, 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 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 χ^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 (size at the end/size at the beginning)/(end date − initial date)], measured in units of 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 *et al.,* 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-populational differences were significant for all morphological variables, highland females being larger than lowland ones. However, independently of inter-populational 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).

Table 1. Mean (±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	Striped	Reticulated-blotched	Striped	Female morph		Population	
	(*n* = 21)	(*n* = 41)	(*n* = 26)	(*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**

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 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).

*Table 2. Mean (±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	Striped	Female morph		Population	
	(*N* = 31)	(*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^{-1} * 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

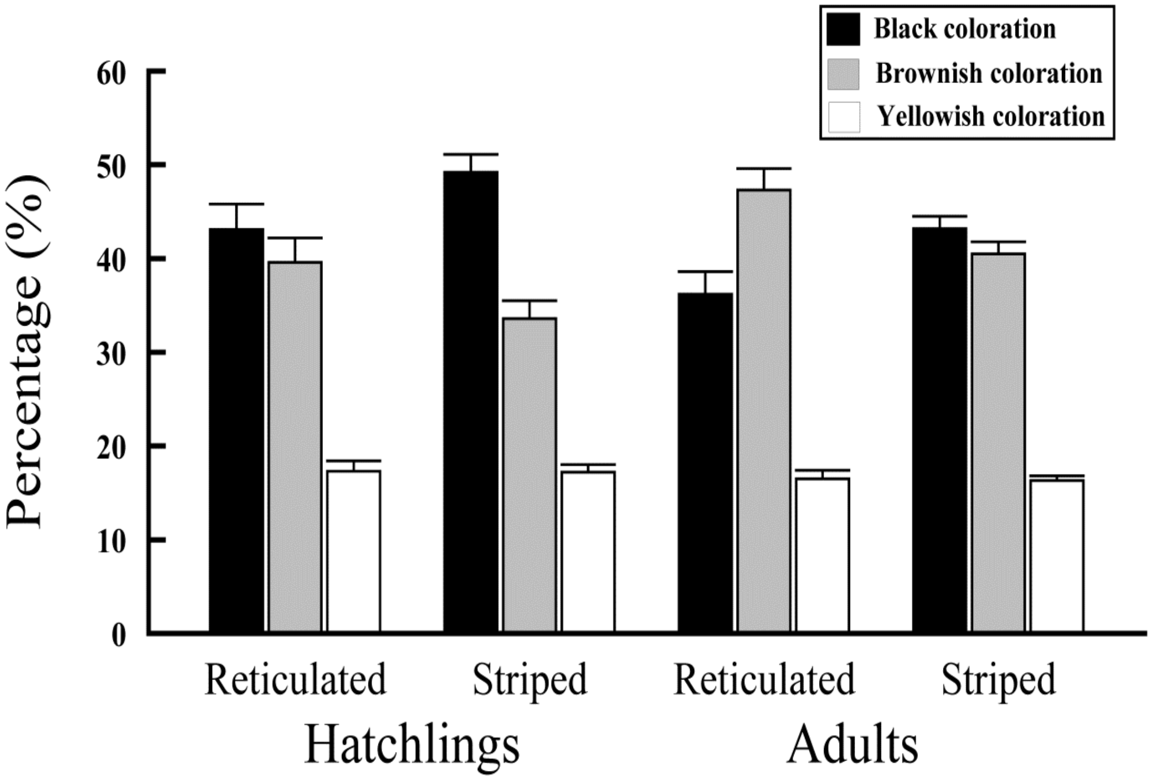

Figure 1. Mean (± 1 SE) values for dorsal coloration traits in hatchling and adult female P. hispanicus from the two female morphs.

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.

Table 3. Mean (±1 SE) growth rates (days^{-1} x 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 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

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 constrains 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 *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, 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 *et al*., 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 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 *et al.,* 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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Chapter V

Altitudinal variation in morphology and coloration in the brown lizard (*Podarcis liolepis*)

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Abstract: Widespread species that occupy different environments often show extensive phenotypic variation and organisms distributed over a wide altitudinal range may be locally adapted, generating clines for several traits with altitude. In this work, we examined altitudinal variation in *P. liolepis* in southwestern France. We found that highland *P. liolepis* lizards were larger, stockier and had more femoral pores and a darker dorsal coloration than lowland ones. Thus, highland and lowland *P. liolepis* follow the same pattern of variation observed in other species within the *P. hispanicus* species complex. Dorsal and ventral coloration fluctuate seasonally and between populations. Brightness of ventral and dorsal coloration are higher in lowland than in highland lizards in spring whereas the reversed trend is found in summer. In addition, throat bluish reflectance is higher in summer than in spring and is also higher in males for all body regions except for the throat. We determine the existence of an anterior-posterior gradient of red ventral coloration, with the highest values in the throat. In addition, brownish coloration was higher in summer than in spring whereas greenish coloration was higher in spring than in summer lizards.

Keywords: coloration, altitudinal variation, morfology, femoral pores, *P. liolepis*

Introduction

Widespread species that occupy different environments often show extensive phenotypic variation. Understanding patterns of geographical variation has focused the interest of scientists as the mechanisms producing intraspecific variation are qualitatively similar to those responsible for macroevolutionary patterns (Dobzhansky, 1937; Mayr, 1942, 1963).

Much progress in understanding the links between environmental and phenotypic variation has been made through the study of species distributed over steep environmental gradients, such as latitudinal or altitudinal clines (Sears & Angilletta 2003; Stillwell, 2010). The ecology of ectotherms should be particularly affected by altitude because so much of their biology is temperature dependent (Huey, 1982) and temperature is negatively correlated with altitude (Yoshino, 1975). Thus, organisms distributed in a wide altitudinal range may be locally adapted, generating clines for several traits with altitude (Welter-Schultes, 2000; Blackburn & Ruggiero, 2001; Ashton & Feldman, 2003; Reguera *et al.,* 2014). Studies on geographical variation in body size have commonly focused on testing Bergmann's rule (Blackburn *et al.,* 1999; Millien *et al.,* 2006; Gaston *et al.,* 2008). Research in reptiles has yielded contrasting results. Results are puzzling as many squamates exhibit reversed Bergmann´s clines but different trends can be found within a genus (Ashton & Feldman, 2003; Sears, 2005) or even within the same species (e.g. *Sceloporus undulatus* (Ferguson & Talent 1993; Niewiarowski & Roosenburg, 1993).

Animal sexual signals may vary among species, populations, seasons or sexes, and its evolution results from the balance between natural and sexual selection (Endler, 1978; 1983; Houde, 1997). Moreover, environmental conditions, which vary with geography and microhabitat, may influence signal detectability or efficacy (Endler, 1992; Endler & Basolo, 1998; Espmark *et al.,* 2000; Leal & Fleishman, 2004 Martín & López, 2006).

Divergence of mate signals and preferences likely occurs because geographically distinct populations experience different environmental conditions that can influence the strength or direction of sexual selection (Endler, 1977). In lizards, the condition dependence and sexually selected nature of carotenoid- and pteridin-based ventral coloration suggest that these ornaments would be particularly variable among populations (Cooper & Greenberg, 1992; Kwiatkowski & Sullivan, 2002).

There are three main hypotheses to account for altitudinal variation in dorsal coloration. The 'thermal melanism hypothesis' relies on the assumption that darker individuals have thermoregulation advantages (fast heating rates) and hence, we should find them in habitats with low temperatures such as highland populations (Watt, 1969; Clusella-Trullas *et al.,* 2007). According with the 'protection against UV damage hypothesis' (Porter & Norris, 1969) darker individuals are expected at higher elevations due to the protection of melanins against the harmful effects of UV radiation (e.g. DNA damage and cellular oxidative stress (Ravanat *et al.,* 2001; Chang & Zheng, 2003), which are stronger at higher altitude. Finally, coloration conspicuousness is a combined function of ambient light and background reflectance and it is ultimately determined by altitude among other factors (Stuart-Fox *et al.,* 2007). Thus, predation may select coloration that render individuals inconspicuous against their visual backgrounds, favouring cryptic coloration (Endler, 1984; Merilaita *et al.,* 1999; Stuart-Fox *et al.,* 2004).

Podarcis hispanicus is a widespread species complex in which genetic and phenotypic variation are extremely high both within and among lineages or species (Sá-Sousa *et al.,* 2002; Pinho et al. 2007; Kaliontzopoulou *et al.,* 2011; Carretero 2008; Renoult *et al.,* 2009; Kaliontzopoulou *et al.,* 2012). In the present work, we tested the hypothesis that morphology, dorsal and ventral coloration and femoral secretions vary with altitude in *P. liolepis*. Other lacertid lizard species are larger at higher altitudes (Zamora-Camacho *et al.,* 2014; Ortega *et* *al.,* 2014) and have more femoral pores (Gabirot *et al.,* 2012). In a similar fashion, it has been shown that lizards are darker as the altitude increases (Reguera *et al.,* 2014). Thus we predict that *P. liolepis* inhabiting highland sites would be larger, darker and would have more femoral pores than in the lowlands as this pattern has also been found in *P. guadarramae,* which is part of this species complex (Gabirot *et al.,* 2012; Gabirot *et al.,* 2013; Ortega *et al.,* 2014).

We specifically examined morphology and dorsal and ventral coloration of three *P. liolepis* populations distributed along an altitudinal gradient in the French Pyrenees.

Material and methods

Study species

The Brown lizard *Podarcis liolepis* (Boulenger, 1905) is a small lacertid that has been recently named and described as a species within the *Podarcis hispanicus* species complex (Renoult *et al.,* 2009, 2010). It is distributed along the Mediterranean Spanish coast from Valencia to Catalonia, westwards to the Basque Country, the Ebro Valley and the Castilian plateau; and in south-east France down the Rhone river (Carretero *et al.,* 2006; Renoult *et al.,* 2010; Kaliontzopoulou *et al.,* 2011).

During April and September-October 2011 we captured by noosing sexually mature male and female lizards at several population in Ariège, in the region of the Midi-Pyrenees, France. `Pêch de Foix´ (42°57'46.79"N, 1°37'22.17"E; 800 m altitude) and `Col de Chioula´ (42°44'53.20"N, 1°50'25.58"E; 1700 m altitude) populations were both surveyed at the beginning and the end of the breeding season. A third population in `Le Mas d´Azil´ (43° 4'22.17"N, 1°21'29.47"E; 400 m altitude) was surveyed only at the end of the breeding season. Hereafter, we will use the names Foix, Azil (lowland sites) and Chioula (highland site) for brevity. In these populations brown lizards can be found on rock cliffs and boulders, but never associated to human constructions which were occupied by *Podarcis muralis*. Both species live in sympatry but have never been observed in sintopy in these populations (J. Ortega pers. observation).

Morphological measurements

We measured body size of lizards using a rule (snout-vent length, ‘SVL’; to the nearest 1 mm). 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 mean number of femoral pores in the ventral side of left and right thighs.

We used a principal component analysis (PCA) to reduce log-transformed morphological variables, except femoral pore number, to a single component. As a result, a main component (morphology PC-1) was obtained which explained 75.41% of variance (eigenvalue = 3.77) and was negatively and significantly correlated with all biometrical measurements ($r < -0.97$ in all cases). The PC scores for morphology were used as an index of body size and shape in subsequent analyses. Post hoc pairwise comparisons were planned using Tukey’s honestly significant difference tests (Sokal & Rohlf, 1995).

Coloration measurements

We used an Ocean Optics USB 2000 spectrophotometer to measure reflectance of lizard coloration using a dual DT-1000-MINI Deuterium–Halogen light source (Ocean Optics, Inc., Dunedin, FL, USA). A custom-made probe holder oriented at 45 degrees and 1 cm away from the skin surface was used to exclude ambient light and standardize measuring distance. Each spectral reading consisted of percent reflectance recordings in reference to a white standard. Spectral raw data were processed with CLR 1.1. software (Montgomerie, 2009) and reflectance readings from 300 to 700 nm, summarized over 5 nm steps ("binned"; Grill & Rush, 2000), were selected for analysis, as they represent the spectral sensitivity measured for other lizard species (Ellingson *et al.* 1995; Fleishman *et al.,* 1993, 1997; Loew *et al.,* 2002) and their avian predators (Cuthill *et al.,* 1999).

We measured coloration of dorsal and ventral body areas. Dorsal coloration was measured at two mid-dorsal standardized spots: above the scapular hip ('proximal dorsal') and above the pelvic hip ('caudal dorsal'). On the other hand, ventral coloration was measured at four standardized spots: the middle of the throat (between the last chin shields and the collar; 'throat'), the breast (just anterior to the two forelimbs at the middle of the second row of scales prior to the collar; 'breast'), in abdominal area ('belly'; in the middle point between 'breast' and 'precloacal'), and the precloacal area (in the middle point before the two forelimbs; 'precloacal').

Principal components analyses were performed for all males and females in both seasons but separately for dorsal and ventral areas. The PCA summarises all of the information about the shape of complex reflectance spectra, including bimodal ones, such as those found here, into a few PCs independent from each other. In PCA of spectral data, PC1 represents variation in intensity of coloration or brightness, and subsequent PCs represent combinations of hue and chroma (Endler, 1990; Cuthill *et al.,* 1999; Grill & Rush, 2000). Also, the PCA identifies those sections of the spectrum (wavelength regions) that are contributing to the observed variation, independently of their importance in terms of contribution to the total amount of reflectance (Montgomerie, 2009).

We used General Lineal Models (GLMs) with each of the PC scores describing coloration as dependent variables and with 'population', 'season' (beginning vs. end of the breeding season) and 'body region' ('proximal dorsal' and 'caudal dorsal', and 'throat', 'chest', and 'precloacal' for dorsal and ventral coloration respectively) as within-subject factor, and including the interactions between effects in the models. We used the Fisher´s LSD test in post hoc pairwise comparisons (Sokal & Rohlf, 1995).

Results

Morphology

Overall, highland lizards, both males and females, were bigger, had more robust heads and longer femora than those from the two lowland populations, as it was for males compared to females in all populations (GLM for PC-1 scores for morphology: sex: $F_{1,63} = 46.23$, $P < 0.001$; population: $F_{2,63} = 6.32$, $P = 0.003$; sex x population: $F_{2,63} = 5.2$, $P = 0.598$; Table 1) (Tukey´s post hoc comparisons: Foix - Chioula: $P < 0.001$; Foix – Azil: $P = 0.783$, Chioula – Azil: $P < 0.001$). These differences were not caused by a larger body length of highland lizards, as adding SVL as a covariate did not change significance, which indicated that highland lizards, and males from both populations were also more robust (GLM with SVL as a covariate: SVL: $F_{1,62} = 256.23$, $P < 0.001$; sex: $F_{1,62} = 117.51$, $P < 0.001$; population: $F_{2,63} = 4.67$, $P < 0.001$; sex x population: $F_{2,63} = 2.56$, $P = 0.085$). Femoral pore number was significantly greater in highland than in lowland lizards of both sexes, and in both populations males had significantly more femoral pores than females (GLM: sex: $F_{1,63} = 46.23$, $P < 0.001$; population: $F_{2,63} = 6.32$, $P = 0.003$; sex x population: $F_{2,63} = 5.2$, $P = 0.598$; Table 1) (Tukey´s post hoc comparisons: Foix - Chioula: $P < 0.001$; Foix – Azil: P =0.499 , Chioula – Azil: $P < 0.001$). These differences remained significant even after controlling for body size and femur length differences between populations (GLM with relative femur length: relative femur length: $F_{1,62} = 0.10$, $P < 0733$; sex: $F_{1,62} = 13.0$, $P < 0.001$; population: $F_{2,63} = 4.67$, $P = 0.009$; sex x population: $F_{2,63} = 0.90$, $P = 0.409$).

Table 1. Mean (±1 SE) values for morphological variables of adult Podarcis liolepis lizards from highland and lowland populations.

	Pêch de Foix		Col de Chioula		Le Mas d´Azil	
	Females $n = 15$	Males $n = 22$	Females $n = 5$	Males $n = 13$	Females $n = 6$	Males $n = 8$
SVL (cm)	50.2 ± 1.2	52.4 ± 1.0	55.4 ± 2.1	58.0 ± 1.3	50.3 ± 1.9	54.3 ± 1.7
Head length (mm)	9.4 ± 0.3	11.3 ± 0.3	8.3 ± 0.6	12.3 ± 0.4	9.1 ± 0.5	11.1 ± 0.5
Head width (mm)	5.8 ± 0.2	7.1 ± 0.1	6.3 ± 0.3	7.8 ± 0.2	5.6 ± 0.2	6.9 ± 0.2
Head heigh (mm)	4.1 ± 0.1	4.7 ± 0.1	4.3 ± 0.2	5.3 ± 0.1	3.8 ± 0.2	4.6 ± 0.1
Femoral length (mm)	7.3 ± 0.2	8.7 ± 0.2	8.2 ± 0.4	10.1 ± 0.2	7.1 ± 0.3	8.7 ± 0.3
Femoral pore number	16.2 ± 0.2	17.1 ± 0.2	16.7 ± 0.4	18.2 ± 0.3	16.1 ± 0.4	16.6 ± 0.3

Ventral coloration

The PCA on reflectance data of all spectra of ventral coloration produced three principal components (PCs). that together accounted for 95.30% of the variation in the original spectra. The first PC (ventral-PC1) accounted for 71.52 % of variation (eigenvalue = 57.93). Coefficients relating ventral-PC1 to the original reflectance data were all negative and of similar magnitude, so ventral-PC1 represented achromatic brightness variation in the original spectra. The second PC (ventral-PC2) accounted for a further 19.38 % of the variation (eigenvalue = 15.70) in the original spectra and the pattern of coefficients suggested that positive values represented variation in both short and medium (410-575 nm) wavelengths while negative values reflect variation in both very short (300–405 nm) and very long (580–700 nm) wavelengths. Thus ventral-PC2 represented variation in the relative amount of both very short, UV, and very long wavelengths, to medium wavelength reflectance, with greater ventral-PC2 scores indicating more saturated ‘greenish’ colors. The third PC (ventral-PC3) accounted for 4.40 % of the variation (eigenvalue = 3.56). The coefficients relating the PC3 to the original reflectance values below 510 nm were all positive, while above 510 nm they were negative. Thus, more negative ventral-PC3 scores indicated more saturated‘orange’ colours.

Initial GLM models showed that the effects of the interactions involving fixed factors described by the three ventral-PCs were not significant, except in the ventral-PC1 for the population x season interaction (all P′s > 0.081). Thus, we excluded these interactions from further analyses. Brightness (ventral-PC1) differed between body regions and the population x season, body region x season and body region x sex interactions were also significant (GLM: population: $F_{1,46} = 2.55$, $P = 0.117$; season: $F_{1,46} = 0.15$, $P = 0.703$; sex: $F_{1,46} = 0.15$, $P = 0.704$; population x season: $F_{1,46} = 24.40$, $P < 0.001$; body region: $F_{3,138} = 8.99$, $P < 0.001$, body region x population: $F_{3,138} = 5.78$, $P = 0.507$; body region x season: $F_{3,138} = 4.73$, $P =$

0.004; body region x population x season: $F_{3,138}$= 0.07, P = 0.978). Hence, the breast had the lowest brightness (Tukey post hoc test: P < 0.001 in all comparisons), while the other body regions showed similar values (P´s > 0.094 in all cases). In addition, lowland lizards were brighter than highland ones in spring whereas highland lizards were brighter than lowland ones in summer, males had less brightness than females in all body regions except for the throat which was similar between sexes and throat was brighter in spring whereas the other body regions showed similar brightness throughout the year. Except for body region all the interactions remained significant after controlling for body size (GLM with SVL as a covariant: SVL: $F_{1,45}$ = 1.73, P = 0.195; population x season: $F_{1,45}$ = 25.14, P <0.001; body region: $F_{3,135}$ = 0.68, P = 0.565; body region x season: $F_{3,135}$= 3.63, P = 0.015; body region x sex: $F_{3,135}$= 4.15, P = 0.008; body region x SVL: $F_{3,135}$ = 0.68, P = 0.563; body region x population x season: $F_{3,135}$ = 0.06, P = 0.980).

The ventral-PC2 showed significant differences in body region, body region x season and body region x sex interactions (GLM; population: $F_{1,47}$ = 0.54, P = 0.467; season: $F_{1,47}$ = 0.05, P = 0.829; sex: $F_{1,47}$ = 0.47, P = 0.493, body region: $F_{3,141}$ = 8.96, P < 0.001, body region x population: $F_{3,141}$ = 0.75, P = 0.524; body region x season: $F_{3,141}$ = 5.75, P < 0.001; body region x sex: $F_{3,141}$ = 4.45, P = 0.005). Thus, the breast reflected more bluish radiation than the belly (Tukey post hoc test: P = 0.003) and the throat reflected more bluish radiation than the belly and the precloacal region (P < 0.001 and P = 0.029, respectively) while the rest comparisons were not significant (all *P´s* > 0.113). However after controlling for body size only the body region x season and body region x sex interactions remained significant (GLM with SVL as a covariant: SVL: $F_{1,46}$= 0.03, P = 0.870; body region: $F_{3,138}$ = 1.82, P = 0.147, body region x population: $F_{3,135}$ = 1.98, P = 0.119; body region x season: $F_{3,138}$ = 4.32, P = 0.006; body region x sex: $F_{3,138}$ = 5.14, P = 0.002, body region x SVL: $F_{3,138}$ = 1.94, P = 0.126). So bluish radiation reflectance was higher in males for all body regions except for the throat which was similar between sexes. Also the throat bluish reflectance was higher in summer than in spring.

Population, season and body region showed significant differences in ventral-PC3 (GLM; population: $F_{1,47} = 4.65$, $P = 0.036$; season: $F_{1,47} = 4.94$, $P = 0.031$; sex: $F_{1,47} = 2.24$, $P = 0.141$, body region: $F_{3,141} = 38.61$, $P < 0.001$, body region x population: $F_{3,141} = 0.72$, $P = 0.540$; body region x season: $F_{3,141} = 0.08$, $P = 0.972$; body region x sex: $F_{3,141} = 0.63$, $P = 0.594$). Throat showed more negative ventral-PC3 scores than breast, belly and precloacal (Tukey post hoc test: $P < 0.001$ in all cases), and breast showed more negative ventral-PC3 scores than belly and precloacal (Tukey post hoc test: $P < 0.001$ and $P = 0.044$, respectively), while belly and precloacal differences were not significant ($P = 0.959$). Thus, the throat was more red than at any other ventral position, the breast was more red than belly and precloacal whereas this last two position showed similar red coloration values. Lowland and summer lizards were more red than highland and spring ones. However after controlling for body size only differences between populations remained significant (GLM with SVL as a covariant: SVL: $F_{1,46}= 1.82$, $P = 0.184$; population: $F_{1,46} = 6.52$, $P = 0.014$; season: $F_{1,46} = 2.91$, $P = 0.095$; sex: $F_{1,46} = 1.42$, $P = 0.239$; body region: $F_{3,138} = 0.20$, $P = 0.895$, body region x population: $F_{3,138} = 0.41$, $P = 0.746$; body region x season: $F_{3,138} = 0.15$, $P = 0.928$; body region x sex: $F_{3,138} = 0.54$, $P = 0.658$; body region x SVL: $F_{3,138} = 0.19$, $P = 0.901$).

Dorsal coloration

The PCA on reflectance data of all spectra of dorsal coloration produced three principal components (PCs) that together accounted for 97.65% of the variation in the original spectra . The first PC (dorsal-PC1) accounted for 85.19% of variation (eigenvalue = 69.00). Coefficients relating dorsal-PC1 to the original reflectance data were all negative and of similar magnitude, so dorsal-PC1 represented achromatic brightness variation in the original spectra. The second PC (dorsal-PC2) accounted for a further 11.23% of the variation (eigenvalue = 9.09) in the original spectra and the pattern of coefficients suggested that positive values represented variation in both short and medium (415-610 nm) wavelengths while negative values reflect variation in both very short (300–410 nm) and very long (615–700 nm) wavelengths. Thus dorsal-PC2 represented variation in the relative amount of short, UV to long, visible, wavelength reflectance, with greater dorsal-PC2 scores indicating more 'greenish' coloration. The third PC (dorsal-PC3) accounted for 1.23% of the variation (eigenvalue = 1.00). The coefficients relating dorsal-PC3 to the original reflectance values below 535 nm were all positive, while above 535 nm they were negative. Thus, more negative dorsal-PC3 scores indicated more saturated 'brownish' dorsal coloration.

Preliminary analysis showed that dorsal coloration did not differ between proximal and caudal regions (all P′s > 0.574) thus we averaged principal component scores for these regions. Initial GLM models showed that the effects of the interactions involving fixed factors described by the three PCs were not significant, except in the dorsal-PC1 for the population x season interaction (all P′s > 0.248). Thus, we excluded these interactions from further analyses. Brightness (dorsal-PC1) did not significantly differ between populations, seasons, sexes or body region but the population x sex interaction was significant (GLM; population: $F_{1,46}$= 3.25, $P = 0.078$; season: $F_{1,46}$= 2.93, $P = 0.094$; sex: $F_{1,46}$= 0.38 $P = 0.542$; population x season interaction: $F_{1,46}$= 44.59, $P < 0.001$). According to these, lowland lizards were brighter than highland ones in spring whereas highland lizards were brighter than lowland ones in summer and this results remained significant after controlling for body size (GLM with SVL as a covariant: SVL: $F_{1,45}$= 1.26, $P = 0.267$; population: $F_{1,45}$= 1.15, $P = 0.288$; season: $F_{1,45}$= 1.66, $P = 0.204$; sex: $F_{1,45}$= 0.70 $P = 0.405$; population x season: $F_{1,45}$= 45.24, $P < 0.001$). However, dorsal-PC2 showed significant differences between seasons (GLM; population:

$F_{1,47}$= 3.76, P = 0.059; season: $F_{1,47}$= 18.28 P < 0.001; sex: $F_{1,47}$= 0.83 P = 0.368). Thus, lizards sampled in spring had more dorsal greenish coloration than summer lizards even after controlling for body size (GLM with SVL as a covariant: SVL: $F_{1,46}$= 0.05, P = 0.824; population: $F_{1,46}$= 2.51, P = 0.120; season: $F_{1,46}$= 15.60, P < 0.001; sex: $F_{1,46}$= 0.69 P = 0.409). The dorsal-PC3 showed significant differences between seasons and populations, thus summer and highland lizards showed more brownish dorsal coloration than spring and lowland ones (GLM; population: $F_{1,47}$= 7.97, P = 0.007; season: $F_{1,47}$= 11.10, P = 0.002; sex: $F_{1,47}$= 0.53 P = 0.470) even after controlling for body size (GLM with SVL as a covariant: SVL: $F_{1,46}$= 1.84, P = 0.182; population: $F_{1,46}$= 9.93, P = 0.003; season: $F_{1,46}$= 13.08, P < 0.001; sex: $F_{1,46}$= 1.01 P = 0.321).

Discussion

The work presented here shows that highland *P. liolepis* were larger and stockier than lowland ones. This result agree with our previous work within the *P. hispanicus* species complex where *P. guadarramae* populations followed Bergmann´s rule (Ortega *et al.,* 2014) (i.e. larger body size in colder environments). It is worth noting that *P. liolepis* and *P. guadarramae* belong to different lineages recently described as distinct species (Renoult *et al.,* 2010; Geniez *et al.,* 2014) and they inhabit very different climatic areas, mediterranean-oceanic versus mediterranean-continental, respectively. Thus, deviations from the reversed Bergmann´s clines, as opposed to the vast mayority of squamates (Ashton & Feldman, 2003), might be the general trend in this species complex. In addition, highland *P. liolepis* had more femoral pores than those from the lowlands, a pattern of variation also present in *P. guadarramae* (Gabirot *et al.,* 2012). It is assumed that femoral pore number reflects an investment in and the use of chemical communication (Escobar *et al.,* 2001; Pincheira-

Donoso *et al.,* 2008). In the context of communication optimization, selection may adjust femoral pore number depending on the influence of the physical conditions (e.g. chemical affinity) of substrate and climatic conditions (e.g. temperature and humidity) on signal detectability and persistence (Alberts, 1992; Escobar *et al.,* 2003; Pincheira-Donoso *et al.,* 2008). Nonetheless, a recent study found little support for the hypothesis that climate conditions co-vary with the diversification of femoral pore number across lacertid lizards (Baeckens *et al.,* 2014). However, we think that focusing on the whole Lacertidae family at the same time could obscure the underlying relationships at lower taxonomic units. Thus, the study of a wide spread species over different climatic regions might help to draw more accurate conclusions.

In terms of ventral brightness, lowland lizards were brighter than highland ones in spring whereas the reversed relationship was found in summer. In addition, throat bluish reflectance was higher in summer than in spring. *P. liolepis*, as other lacertid lizards shows a carotenoid-based ventral polymorphism in both sexes (J. Ortega pers. observ.). This kind of polymorphism has been linked, in lacertids, as in many other squamates, to behavioural, reproductive and physiological differences between morphs (Sinervo, 2001; Svensson *et al*., 2001; Vercken *et al*., 2007). Moreover, body coloration may function as a sexual ornament or as an status signal (Olsson, 1994; Stuart-Fox *et al.,* 2006) which may explain why bluish radiation reflectance was higher in males for all body regions except for the throat which was similar between sexes. Evolutionary divergence in signal characteristics that improve the efficiency of communication might occur if members of related taxa consistently experience different environmental conditions across many generations (e.g. Derryberry, 2007). In this case, habitat openness or vegetation cover affect light conditions, which may exert strong selection on visual signals such as ventral coloration to maximize conspicuousness (Marchetti, 1993; Endler & Thery, 1996; Zahavi & Zahavi, 1997; Andersson, 2000) and lead to an increase in the signal-to-noise ratio, or intensity, of communication by changing the structural properties of signals (Ord *et al.,* 2010). Thus, prevailing light conditions may render some ventral coloration variations more effective than others in highland vs lowland habitats, but also between spring and summer, as seasonal variation in body coloration may occur in animals with a distinct reproductive season (Carretero, 2002; Germano & Williams, 2007). We also determined the existence of a ventral gradient of red coloration, with the highest values in the throat and the lowest ones in belly and precloacal. This result is in line with the interpretation that the ventral position of these areas means that they remain hidden except when the lizard raises its forebody in the presence of a conspecific (Pérez-Mellado & Galindo-Villardón, 1986; Galán, 1995).

With respect to dorsal coloration, lowland lizards were brighter than highland ones in spring whereas the reversed trend was found in summer. This is in accordance with a thermoregulation hypothesis in which the lower temperatures of high altitude sites would favour darker dorsal coloration, as darker reptiles have several thermoregulation advantages such as fast heating rates and higher thermal inertia (Majerus, 1998; Bittner *et al.*, 2002). Hence, lowland lizards might have a higher dorsal reflectance at the beginning of the breeding season (spring) as the thermal conditions of their habitat are less restrictive than in the highlands. The same reasoning is argued by Gabirot *et al.*(2013b) for *P. guadarramae* where they found that highland lizards were darker than lowland ones. Lowland lizards also had more greenish dorsal coloration than highland ones and summer lizards. In a similar fashion, summer lizards, with independence of its population of origin, had more brownish coloration. Most evidence shows that dorsal coloration is generally presumed to be an adaptation for crypsis or thermoregulation in diurnal reptiles (Norris, 1965; Kettlewell, 1973, Rosenblum, 2005) and background matching has been widely documented in reptiles (King, 1992; Macedonia *et al.,* 2002; Bittner, 2003). Seasonal variation in body coloration may also occur in species living in habitats where background colours (Wente & Phillips, 2005) or the thermal environment (Fields & McNeil, 1988) change with the time of year. Thus, background matching might be responsible for these changes in dorsal coloration, due to microhabitat differences (e.g. vegetation). or differential habitat selection between populations and seasons. It is worth noting that ventral and dorsal brightness are correlated among populations and between seasons, so we should not dismiss that both traits could be linked, and hence, brightness should be considered as a whole without asking for adaptive explanations separately.

Another factor that may affect dorsal and ventral coloration is predation. Predators may exploit signals to localize prey (Endler, 1980; Ryan, 1987), and habitat characteristics, such as openness, can in turn determine the vulnerability of signallers to visual predators. Habitat type, through its influence both on properties of the signalling environment and predation pressure, can thus play an important role in the evolution of conspicuous male coloration and ornamentation (Endler, 2000), and hence, be responsible for interpopulation coloration differences.

In summary, our results indicated that highland *P. liolepis* were larger, stockier and had more femoral pores and a darker dorsal coloration than lowland ones darker dorsal coloration than lowland ones, a pattern of variation observed in other species within the *P. hispanicus* species complex. We found seasonal and population variation in both dorsal and ventral coloration. Ventral and dorsal brightness was higher in lowland than in highland lizards in spring whereas the reversed trend was found in summer. In addition, summer lizards had more throat bluish reflectance than spring ones. Males also had more throat bluish reflectance in all body regions except for the throat. We determined the existence of an anterior-posterior gradient of red coloration, with the highest values in the throat. In addition, summer lizards had more brownish coloration than spring ones whereas spring lizards had more greenish coloration than summer ones. Our results highlight that high phenotypic variation can be present even in closely related population as genetical analyses of the ND4 mitochondrial marker showed that all the populations studied here are true *P. liolepis* and belong to the same lineage (Ortega *et al.,* unpub. results).

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Chapter VI

This chapter reproduces entirely the manuscript:

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Interpopulational Variations in Sexual Chemical Signals of Iberian Wall Lizards May Allow Maximizing Signal Efficiency Under Different Climatic Conditions

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Abstract: Sexual signals used in intraspecific communication are expected to evolve to maximize efficacy under a given climatic condition. Thus, chemical secretions of lizards might evolve in the evolutionary time to ensure that signals are perfectly tuned to local humidity and temperature conditions affecting their volatility and therefore their persistence and transmission through the environment. We tested experimentally whether interpopulational altitudinal differences in chemical composition of femoral gland secretions of male Iberian wall lizards (*Podarcis hispanicus*) have evolved to maximize efficacy of chemical signals in different environmental conditions. Chemical analyses first showed that the characteristics of chemical signals of male lizards differed between two populations inhabiting environments with different climatic conditions in spite of the fact that these two populations are closely related genetically. We also examined experimentally whether the temporal attenuation of the chemical stimuli depended on simulated climatic conditions. Thus, we used tongue-flick essays to test whether female lizards were able to detect male scent marks maintained under different conditions of temperature and humidity by chemosensory cues alone. Chemosensory tests showed that chemical signals of males had a lower efficacy (i.e. detectability and persistence) when temperature and dryness increase, but that these effects were more detrimental for signals of the higher elevation population, which occupies naturally colder and more humid environments. We suggest that the abiotic environment may cause a selective pressure on the form and expression of sexual chemical signals. Therefore, interpopulational differences in chemical profiles of femoral secretions of male *P. hispanicus* lizards may reflect adaptation to maximize the efficacy of the chemical signal in different climates.

Keywords: Chemical signals, *Podarcis hispanicus*, adaptation, temperature, humidity

Introduction

Animals use sexual signals to inform conspecifics on different traits of the sender (Bradbury & Vehrencamp, 2011). In many cases, these sexual signals evolve to ensure reliability of the message, and also to maximize signal efficacy in a given environment (Bradbury & Vehrencamp, 2011, Endler & Basolo, 1998). The efficacy of a signal depends on factors such as how well the signal is transmitted through the environment, durability or persistence of the signal, or how well the signal is detected by the sensory system of receivers. These factors will determine how selection shapes the characteristics of the signal in order to maximize perception by the receiver (Guilford & Dawkins, 1998; Boughman, 2002). Darwinian selection may maximize the efficacy of sexual signals in different environments or under different climatic conditions (Bradbury & Vehrencamp, 2011; Boughman, 2002).

Chemoreception is one of the main sensory systems for many animals, including many vertebrates, and chemical signals play an important role in communication and sexual selection in these animals (Müller-Schwarze, 2006; Wyatt, 2014). For example, in lizards, chemical signals from femoral glands or faeces are very often used to scent-mark substrates allowing to delimit territories or attract mates (Mason, 1992; Martín & López, 2012). Chemical signals in scent-marks may provide information on sex, size, dominance, and even details of health condition of the signaller (Mason & Parker, 2010; López *et al.,* 2006; Martín & López, 2015). This information is important in intrasexual relationships between males (Martín & López, 2002; Martín *et al.,* 2007) and in female mate choice (López & Martín, 2005; Kopena *et al.,* 2011). Therefore, chemical signals are expected to evolve to honestly signal traits involved in sexual selection (Martín & López, 2015).

In addition, chemical signals used for scent marking should ensure efficiency by appropiately tuning to environmental factors such as local humidity and temperature (i.e.

affecting their volatility and therefore their persistence and transmission through the environment) (Alberts, 1992; McDonough *et al.,* 1989; Regnier & Goodwin, 1977). Thus, it has been suggested that the observed interspecific or interpopulational differences in chemical signals of lizards might partly reflect selection for maximizing the efficacy of signals under different climatic conditions) (Alberts, 1992; Escobar *et al.,* 2001; Gabirot et al., 2012a]. However, this hypothesis has never been tested experimentally.

In this paper, we tested whether interpopulational differences in chemical composition of femoral gland secretions of male lizards may have evolved to maximize efficacy of chemical signals under different environmental conditions. We examined experimentally the effects of climatic conditions on the persistence and efficacy of chemical sexual signals of Iberian wall lizards (*Podarcis hispanicus*). This is a small lacertid lizard living in rocky habitats of the Iberian Peninsula. Males scent-mark substrates with femoral gland secretions, which contain a mixture of proteic and lipophilic compounds, mainly steroids, fatty acids, alcohols, and waxy esters (Martín &López, 2006b; Gabirot *et al.,* 2013a). Chemical signals are very important in intraspecific relationships of this lizard (Martín &López, 2001). Males gauge scent-marks of other males to identify potential rivals and assess their competitive ability (Martín & López, 2002; Carazo *et al.,* 2007; 20]. Females show strong chemosensory responses and prefer areas scent-marked by males that allocate more cholesta-5,7-dien-3-ol to femoral secretions (López & Martín, 2005; Gabirot *et al.,* 2012a; Gabirot *et al.,* 2013a, Martín & López, 2006a), which are individuals of presumably high quality as suggested by, for example, their more efficient immune response (López & Martín, 2005).

This lizard species exhibits substantial levels of intraspecific phenotypic variation (Carrretero, 2008). Molecular studies suggest that the Iberian wall lizard is paraphyletic and forms part of a species complex with at least five monophyletic lineages and several now well recognised species (Carrretero, 2008; Pinho *et al.,* 2007) But even inside the same genetic lineages, there is an important phenotypic variation between some populations which could be due to the different environments that they occupy. For example, in Central Spain, there are several distinct populations of *P. hispanicus*, with clear phenotypic differences, that occupy nearby areas with very different climatic conditions [Gabirot *et al.,* 2012b; Gabirot *et al.,* 2013b;-43]. Moreover, the chemical composition of femoral secretions of males differs even between closely genetically related populations (Gabirot *et al.,* 2013a). Here, we studied two genetically related populations of *P. hispanicus* lizards from the Guadarrama Mts. (Central Spain) that live at different altitudinal ranges with contrasting climatic differences. We 1) analysed chemical composition of femoral gland secretions of males of both populations using gas chromatography-mass spectrometry (GC-MS) and 2) tested the chemosensory ability of female lizards to detect males' scent marks that were experimentally maintained under different temperature and humidity/dryness regimes. We examined whether the temporal attenuation of the chemical stimuli differed between populations depending on temperature and humidity. We predicted that the efficacy (i.e., detectability and persistence) of scent marks for each population should be maximal under the local environmental conditions of each population, and discussed how this local adaptation may be based on interpopulational differences in compounds in secretions.

Material and Methods

Study animals

We captured adult *P. hispanicus* lizards by noosing during April-May 2012, at two populations at different elevations in the Guadarrama Mountains (Central Spain), separated 6 km by air. The 'lower elevation' locality is an oak forest (`La Golondrina´) near Navacerrada village (40º43´ N, 04º01´ W; 1,190 m altitude), where lizards can be found on granite rocky outcrops inside the forest. The ‘higher elevation’ locality is found in the upper part of `Fuenfría Valley´ near Cercedilla village (40º47´ N, 04º03´ W; 1,750 m altitude) occupying granite rock walls and rock piles at the edge of a pine forest. Analyses of microsatellite data showed a very low genetic divergence between these two populations and a high degree of gene flow, indicating that they belong to the same genetic lineage (Gabirot *et al.,* 2013a)

Lizards were taken to "El Ventorrillo" Field Station, about 5 km away from the sampling sites, the same day of capture. Lizards were kept in two walk-in climatic chambers (Ibercex V-450-D; ASL S.A., Madrid, Spain) where temperature (diurnal = 21 ºC; nocturnal = 15 ºC) and photoperiod (12 h:12 h, light:dark) were controlled automatically. Lizards were individually housed in plastic terraria (40 x 30 x 25 cm) filled with a moistened coconut fibre substrate and provided with a water bowl and a brick (24 x 11 x 8 cm) for shelter and as a basking platform. A 50 W halogen lamp was suspended over one end of the terrarium providing a diurnal temperature gradient (21-45 ºC). This allowed thermoregulation of lizards around the preferred body temperature of this species (34.4 °C) (Bauwens et al., 1995) while ensuring that lizards from each population could freely select different temperatures if there were any small population differences in thermoregulation requirements (Gabirot *et al.,* 2013b). In addition, a fluorescent bulb over each shelf provided ambient lighting mimicking the natural photoperiod, and mercury vapour bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14.00 h to 15.30 h). Lizards were daily watered, and fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*), dusted with a commercial vitamin and calcium supplement, *ad libitum*.

All animals were healthy and were returned to their capture sites at the end of trials. Captures of lizards were performed under license (permit number: 10/072913.9/12) from the Environmental Agency of Madrid Government (“Consejería del Medio Ambiente de la

Comunidad de Madrid”, Spain). Sampling procedures and experimental studies were reviewed and specifically approved as part of obtaining this field permit. The laboratory studies and the husbandry procedure were also approved by the Animal Ethics Committee of the Museo Nacional de Ciencias Naturales (CSIC).

Climatic conditions

We summarised the available environmental temperatures and precipitations in the study areas by using long-term (from 1978 to 2011) daily data from two nearby meteorological stations: ‘Colmenar Viejo’ (40°41’55’’ N, 03°45’52’’ W; elevation 1,004 m; Madrid province) and ‘Puerto de Navacerrada’ (40°46’50’’ N, 04°00’37’’ W; elevation 1,894 m; Madrid province) for the lower and higher elevation population respectively (data available from the web of the Spanish Meteorological Agency, ‘Agencia Española de Metereología, AEMET’; *http://www.aemet.es*). Monthly temperatures were measured as means of daily mean temperatures, and we also calculated means of daily maximum air temperatures, as advised for ecophysiological studies of reptiles (Huey, 1982). We also used total precipitation as an indication of humidity conditions. We used data from April to June, which coincides with the main mating season of lizards, when males have the highest rates of femoral secretions (Martín & López, 2006b).

Chemical signals of lizards

We extracted femoral gland secretion of male lizards from both populations by gently pressing around the femoral pores, and collected secretion directly in glass vials with glass inserts. We also used the same procedure on each sampling occasion, but without collecting secretion, to obtain blank control vials that were treated in the same manner to compare with the actual samples.

Samples of secretions were analyzed using a Finnigan-ThermoQuest Trace 2000 gas chromatograph (GC) fitted with a poly (5 % diphenyl/ 95 % dimethylsiloxane) column (Supelco, Equity-5, 30 m length x 0.25 mm ID, 0.25-µm film thickness) and a Finnigan-ThermoQuest Trace mass spectrometer (MS) as detector. Sample injections (2 µl of each sample dissolved in 200 µl of n-hexane; Sigma, capillary GC grade) were performed in splitless mode using helium as the carrier gas, with injector and detector temperatures at 270°C and 250°C, respectively. The oven temperature program was as follows: 50°C isothermal for 10 min, then increased to 280°C at a rate of 5°C/min, and then isothermal (280°C) for 20 min. Mass spectral fragments below $m/z = 39$ were not recorded. Impurities identified in the solvent and/or the control vial samples were not reported. Initial identification of secretion components was done by comparison of mass spectra in the NIST/EPA/NIH 2002 computerized mass spectral library. When possible, identifications were confirmed by comparison of spectra and retention times with those of authentic standards. Authentic samples were purchased from Sigma-Aldrich Chemical Co.

We identified and calculated relative proportions determined as the percent of the total ion current (TIC) of major compounds (> 1% of the TIC area) in secretions. To compare the compounds found in femoral secretions between the two populations, we used the compositional analysis, consisting in logit transforming the proportion data by taking the natural logarithm of proportion ⁄ (1 – proportion) to correct the problem of nonindependence of proportions (Aebischer *et al.,* 1993). Then, we calculated Euclidean distances between every pair of individual samples to produce a resemblance matrix that formed the basis of the analyses. We used single factor permutational multivariate analysis of variance tests (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001) based on the Euclidean resemblance matrix using 999 permutations to analyze whether the composition of the femoral secretions varied between populations and experimental conditions. Differences were investigated further using canonical analysis of principal coordinates (CAP) (Anderson & Willis, 2003). The software PRIMER V6.1.13 [50] with the PERMANOVA+ V1.0.3 add-on package (Anderson *et al.,* 2008) was used to investigate differences between chemical profiles.

Chemosensory trials

To test for the relative detectability, persistence and efficiency of femoral secretions of males, we compared the chemosensory responses of female lizards in response to scent stimuli arising from cotton applicators bearing femoral secretions of males of their own populations. We examined the effect of different conditions of temperature and humidity (see below) on the temporal fading of the chemical stimuli after the secretions had been deposited on the cotton swabs. Differential rates of tongue-flick (TF) of lizards to different chemical stimuli allow testing detection of chemical cues (Cooper ,1994; Cooper & Burghardt, 1990). We recorded latency to the first TF as an indication of detectability (i.e. more detectable stimuli should have shorter latencies) and numbers of TFs directed to the swab during 1 min, beginning with the first TF, as an indication of the efficiency of the stimuli in eliciting chemosensory responses of females (i.e. more efficient stimuli should elicit higher TF rates because they are supposed to be more attractive) (Martín & López, 2006c, Martín & López, 2006a; Cooper & Burghardt, 1990; Epple *et al.,* 1980). The comparison of responses to the femoral secretions freshly collected from males, and responses to secretions that had spent some time exposed to the environment climatic conditions after they were deposited, allowed testing for temporal persistence of the chemical stimuli in secretions.

We prepared stimuli by taking out femoral secretions of males (n = 24) pressing around the femoral pores and collected the waxy secretion directly on the cotton tip (1 cm) of a wooden applicator. We used approximately the same amount of femoral secretion in each stimulus (2×1 mm of solid secretion from each of three pores) to minimize the likelihood that differences in TF rates were due to differences in the amount of secretion presented. To avoid differences in responses to different individual males (López & Martín, 2005), every female (n = 24; 12 from each population) was always tested with secretions from the same individual male. Secretions used for chemosensory detection tests came from different individual males than those used for chemical analyses (see above) due to limitations in the amount of secretion that a male can produce.

Immediately after, we placed swabs impregnated with chemical stimuli in two incubator chambers at 12 °C and 22 °C ('cold' vs. 'warm', respectively), and left them there for 1 min (initial time), 1 h or 3 h before being used in chemosensory tests. The cold and warm temperatures corresponded approximately to the mean of daily maximum temperatures (those experienced by lizards during their maximum diurnal peak of activity) in the higher and lower elevation study areas respectively during the mating season. The cotton swabs were not in contact with anything inside the chambers, and after being used in a single test were discarded.

Each individual female (twelve from each population) was tested in six treatments: three periods of time since the femoral secretion was deposited x two temperatures, but participated in only one test every day in a random order. Trials were conducted in outdoor conditions during May and between 8:00-12:00 h (GMT) when lizards were fully active. Before the tests, females were allowed to bask and attain an optimal body temperature (around 34.4 °C) (Bauwens *et al.,* 1995). In each trial, the same experimenter (PL), who was blind to the treatment, slowly approached a lizard's home cage and slowly moved the cotton swab applicator attached to a long stick (50 cm) to a position 2 cm anterior to the lizard's snout. Lizards allowed approaching and testing without fleeing. All female lizards responded to swabs by tongue flicking.

In a second experiment, we examined the effect of dryness on the temporal fading of the chemical stimuli after they had been deposited on the cotton swabs under different humidity conditions. We followed a procedure similar to the previous experiment but cotton swabs with femoral secretions of males (*n* = 24; different individuals than in previous tests) were placed in hermetic glass boxes containing either a 2 cm depth water substrate ('humid' treatment), which resulted in a water saturated air inside the box, or silica gel ('dry' treatment), which absorbed humidity and provided a dry environment, during 1 min, 1 h or 3 h. The boxes were placed indoor, protected from light and at the room environment temperature (about 15-17 °C). Chemosensory tests were made as in the previous experiment but using different individual females (*n* = 24; 12 from each population).

To examine differences in latencies or number of directed TFs of the same individual females among treatments, we used repeated measures General Linear Models (LMs) with population (lower *vs*. higher elevation) as a between factor, and time (initial *vs.* 1 h *vs.* 3 h) and temperature (cold *vs.* warm) or dryness treatment (dry *vs*. humid) as within-subjects factors, including the interactions in the models. Data were log-transformed to ensure normality (Shapiro-Wilk's test). Tests of homogeneity of variances (Levene's test) showed that in all cases, variances were not significantly heterogeneous after transformation. Pairwise comparisons used Tukey's honestly significant difference tests (Sokal & Rohlf, 1995). All the statistical analyses were performed with STATISTICA v8.0 (Statsoft Inc., Tulsa, OK, USA).

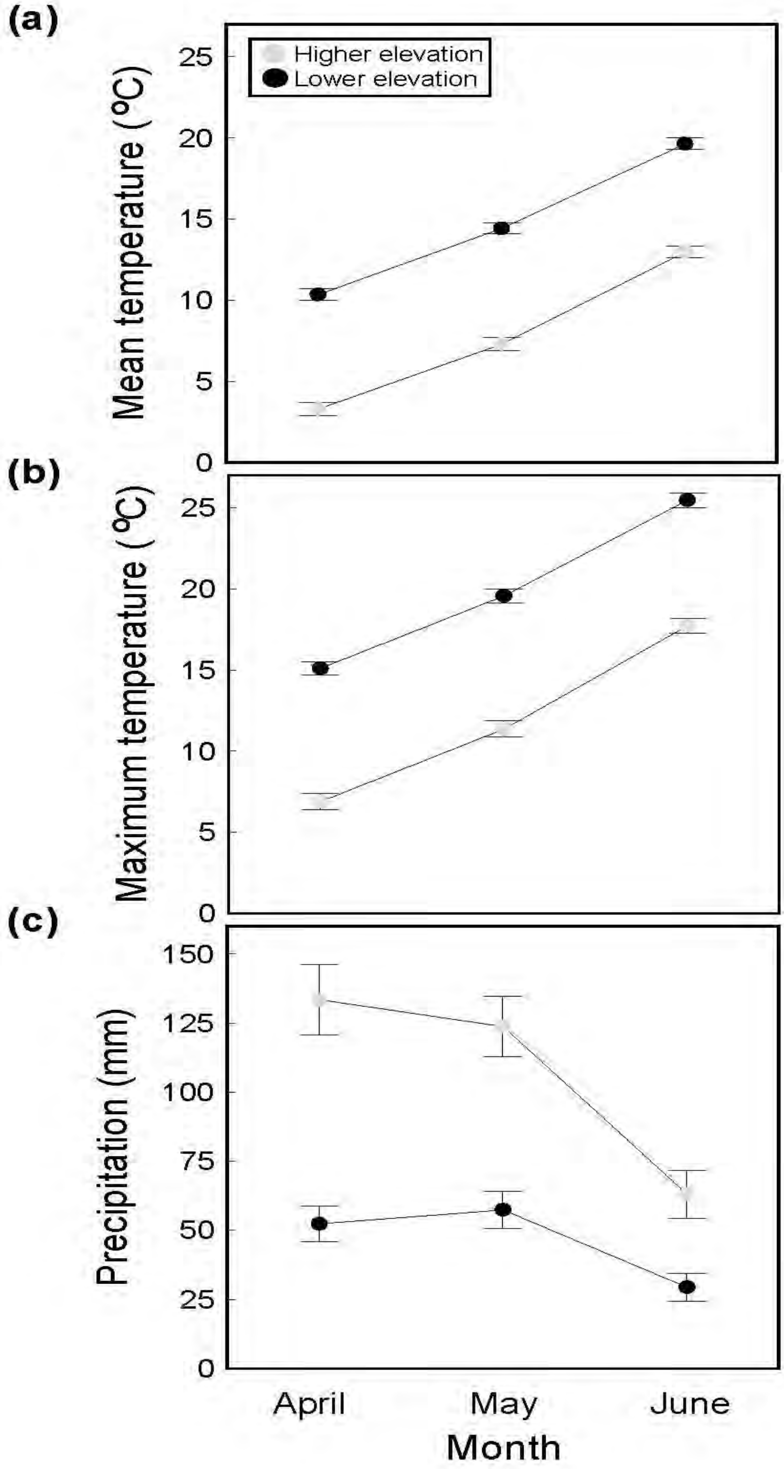

Fig. 1. Climatic Conditions at the Lizards' Populations. Variation in (a) daily mean and (b) daily maximum air temperatures, and (c) total monthly precipitation in two populations (lower elevation: black circles; higher elevation: grey circles) of the Guadarrama Mts., Central Spain, in the months of April, May and June, coinciding with the mating season of Podarcis hispanicus lizards. Data show average (± SE) monthly values for a 34 years period (1978-2011).

Results

Climatic conditions

The mean air temperatures increased significantly within a given year from April to June in both populations but temperatures were significantly greater in the lower elevation population in all these months (two-way ANOVA; month: $F_{2,193} = 328.83$, $p < 0.0001$, partial $\eta^2 = 0.77$; population: $F_{1,193} = 523.62$, $p < 0.0001$, partial $\eta^2 = 0.73$; interaction: $F_{2,193} = 0.22$, $p = 0.80$, partial $\eta^2 = 0.002$) (Fig. 1a). A similar result was observed for the means of daily maximum air temperatures (two-way ANOVA; month: $F_{2,193} = 276{\cdot}02$, $p < 0.0001$, partial $\eta^2 = 0.74$; population: $F_{1,193} = 471.91$, $p < 0.0001$, partial $\eta^2 = 0.71$; interaction: $F_{2,193} = 0.18$, $p = 0.83$, partial $\eta^2 = 0.002$) (Fig. 1b). Total precipitation decreased significantly from April to June in both populations and precipitations were significantly greater in the higher elevation population in April and May, but not in June (two-way ANOVA; month: $F_{2,193} = 17.25$, $p < 0.0001$, partial $\eta^2 = 0.15$; population: $F_{1,193} = 67.71$, $p < 0.0001$, partial $\eta^2 = 0.26$; interaction: $F_{2,193} = 3.67$, $p = 0.0027$, partial $\eta^2 = 0.04$) (Fig. 1c). Therefore, the higher elevation population has a climate that is colder and more humid than in the lower elevation population.

Chemical signals of lizards

Femoral secretions of males from the two populations were formed by similar types of compounds, mainly steroids (83.88%, both populations combined), but also fatty acids between C_8 and C_{24} (10.95%), waxy esters (2.24%), alcohols (1.53%), an aldehyde (0.67%), terpenoids (0.65%), a ketone (0.06%), and a furanone (0.02%). Considering specific compounds, cholesterol (56.2%) was the most abundant one, followed by cholesta-5,7-dien-3-ol (15.9%), hexadecanoic acid (3.0%), octadecenoic acid (2.8%), and ergosta-5,8-dien-3-ol

(2.8%). Both populations shared 50 out of the 61 detected compounds (82%) including all the main ones (i.e. those with proportions > 0.5%). However, there were clear interpopulational differences in proportion of compounds. Thus, the PERMANOVA based on the resemblance matrix comparing the chemical profiles of males from the two populations was statistically significant (pseudo $F_{1,45} = 13.65$, $p < 0.001$). The CAP analysis classified 91.5% of the individual chemical profiles into the correct population using leave-one-out cross-validation and $m = 4$ axes ($\delta_1^2 = 0.65$, $p = 0.001$). Comparing specific types of compounds, lizards from the lower elevation population had significantly higher proportions of cholesterol (LM; $F_{1,45} = 16.43$, $p = 0.0002$) and fatty acids ($F_{1,45} = 12.99$, $p < 0.0008$) but significantly lower proportions of alcohols ($F_{1,45} = 6.09$, $p = 0.017$) than lizards from the higher elevation population. However, there were not significant differences between populations in proportions of cholesta-5.7-dien-3-ol ($F_{1,45} = 0.06$, $p = 0.80$) or other types of compounds, such as waxy esters or terpenoids ($p > 0.30$ in all cases).

Effects of temperature on signal efficacy

The latency to the first TF differed significantly between populations and temperatures and among time periods, and all the two-way interactions were significant (Table 1; Fig. 2a). Females had longer latencies as time since the secretions were deposited increases, and when secretions were previously exposed to warmer temperatures. Therefore, the detectability of the signal decreased with time and at warmer temperatures in both populations. However, the temperature*population and time*population significant interactions showed that these effects (i.e. the greater loss of detectability with time at warm temperature) were more marked in the higher elevation population, where local temperatures were cold. Thus, initial latencies did not significantly differ between populations nor between treatments (Tukey's tests, $p > 0.96$

in all cases), but latencies 1h and 3 h after deposition were significantly longer in the warm than in the cold treatment in both populations (lower elevation: $p = 0.001$; higher elevation: $p = 0.0001$), and, within each temperature treatment, latencies were significantly longer in the higher than in the lower elevation population ($p = 0.0001$ in all cases) (Fig. 2a).

Table 1. Statistical Tests for the Effects of Temperature and Dryness on Signal Efficacy.

		Latency			**Tongue-flicks**		
	df	*F*	*p*	partial η^2	*F*	*p*	partial η^2
Effects of temperature:							
Population	1,22	50.85	<0.0001	0.70	8.19	<0.01	0.27
Temperature	1,22	118.83	<0.0001	0.84	50.33	<0.0001	0.70
Time	2,44	390.87	<0.0001	0.95	360.58	<0.0001	0.94
Temperature x Population	1,22	4.72	0.041	0.18	0.01	0.95	0.0002
Time x Population	2,44	16.56	<0.0001	0.43	0.84	0.44	0.04
Temperature x Time	2,44	17.22	<0.0001	0.44	4.07	0.02	0.16
Population x Temperature x Time	2,44	1.90	0.16	0.08	3.44	0.04	0.13
Effects of dryness:							
Population	1,22	15.93	<0.0001	0.42	13.51	0.0013	0.38
Dryness	1,22	142.38	<0.0001	0.87	52.20	<0.0001	0.70
Time	2,44	431.63	<0.0001	0.95	256.21	<0.0001	0.92

Dryness x Population	1,22	15.82	0.0006	0.42	9.04	0.0065	0.29
Time x Population	2,44	4.01	0.025	0.15	2.85	0.07	0.11
Dryness x Time	2,44	24.48	<0.0001	0.53	4.84	0.013	0.18
Population x Dryness x Time	2,44	4.80	0.013	0.18	0.63	0.54	0.03

Results of full factorial General Linear Models (LMs) examining variation in latencies or number of directed tongue-flicks of the same individual female *P. hispanicus* lizard among treatments, with population (lowler *vs*. higher elevation) as a between factor, and temperature (cold *vs*. warm) or dryness (dry *vs*. humid) treatments and time since deposition (initial *vs*. 1 h *vs*. 3 h) as within-subjects factors. Degrees of freedom (df), *F* statistics, significance level (*p*) and effect size (partial η^2) are shown.

The number of TFs directed by females differed significantly between populations and temperatures, and among time periods, and the temperature*time interaction was significant (Table 1; Fig. 2b). Thus, in both populations, TF rates of females decreased as the time since the secretion was deposited increased and when secretions were previously exposed to warmer temperatures. Therefore, the signal elicited lower responses (was less effective) when time and temperature increased. However, the population* temperature*time triple significant interaction indicated that the combined detrimental effects of temperature and time were more marked in the higher elevation population. Thus, while initial TF rates did not significantly differ between populations (Tukey's tests, $p > 0.67$ in all cases), TF rates 1 and 3 h after deposition did not significantly differ between populations in the cold treatment ($p > 0.87$ in both cases), but in the warm treatment, TF rates 1 and 3 h after deposition were significantly lower in the higher elevation population ($p < 0.05$ in both cases) (Fig. 2b).

Effects of dryness on signal efficacy

The latency to the first TF differed significantly between populations and dryness treatments and among time periods, and all the interactions were significant (Table 1; Fig. 3a). Therefore, females in both populations had significantly longer latencies (i.e. the signal had lower detectability) as the time since the secretion was deposited increased and when secretions were previously exposed to drier conditions. However, the significant interactions showed that these effects (i.e. the greater loss of detectability with time under drier conditions) were more marked in the higher elevation population, where the local climate was more humid. Thus, initial latencies did not differ significantly between populations nor between temperature treatments (Tukey's tests, $p > 0.96$ in all cases), latencies 1 h and 3 h after deposition did not differ between populations in the humid treatment ($p > 0.99$ in both cases), but in the dry treatment latencies were significantly longer in the higher elevation than in the lower elevation population ($p < 0.001$ in both cases) (Fig.3a).

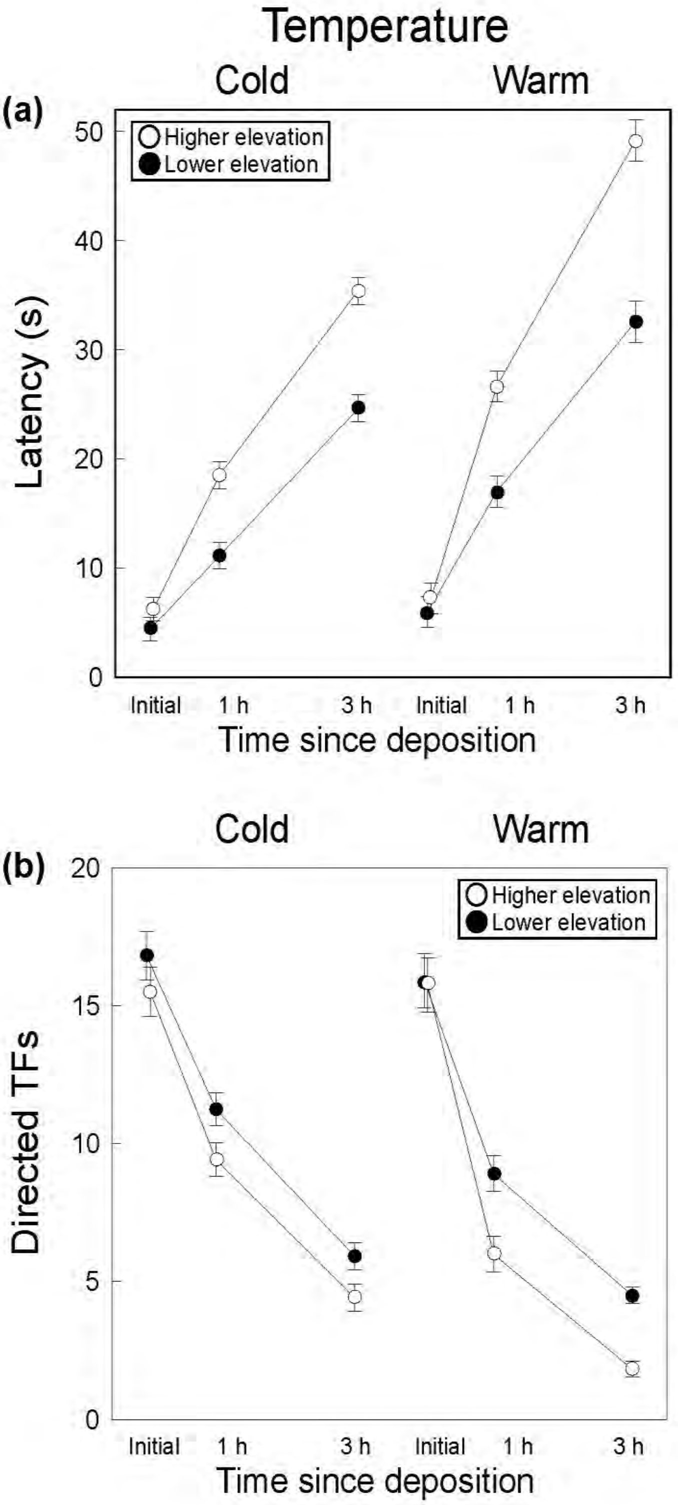

Fig. 2. Effects of Temperature on Signal Efficacy. Mean ± *SE (a) latency and (b) number of tongue-flicks (TF) directed to swabs by female P. hispanicus lizards from two populations (lower elevation: black;higher elevation: white) in response to swabs bearing femoral secretions of males immediately after they were secreted ('initial'), 1 h or 3 h since deposition and maintained under two temperature regimes (cold vs. warm).*

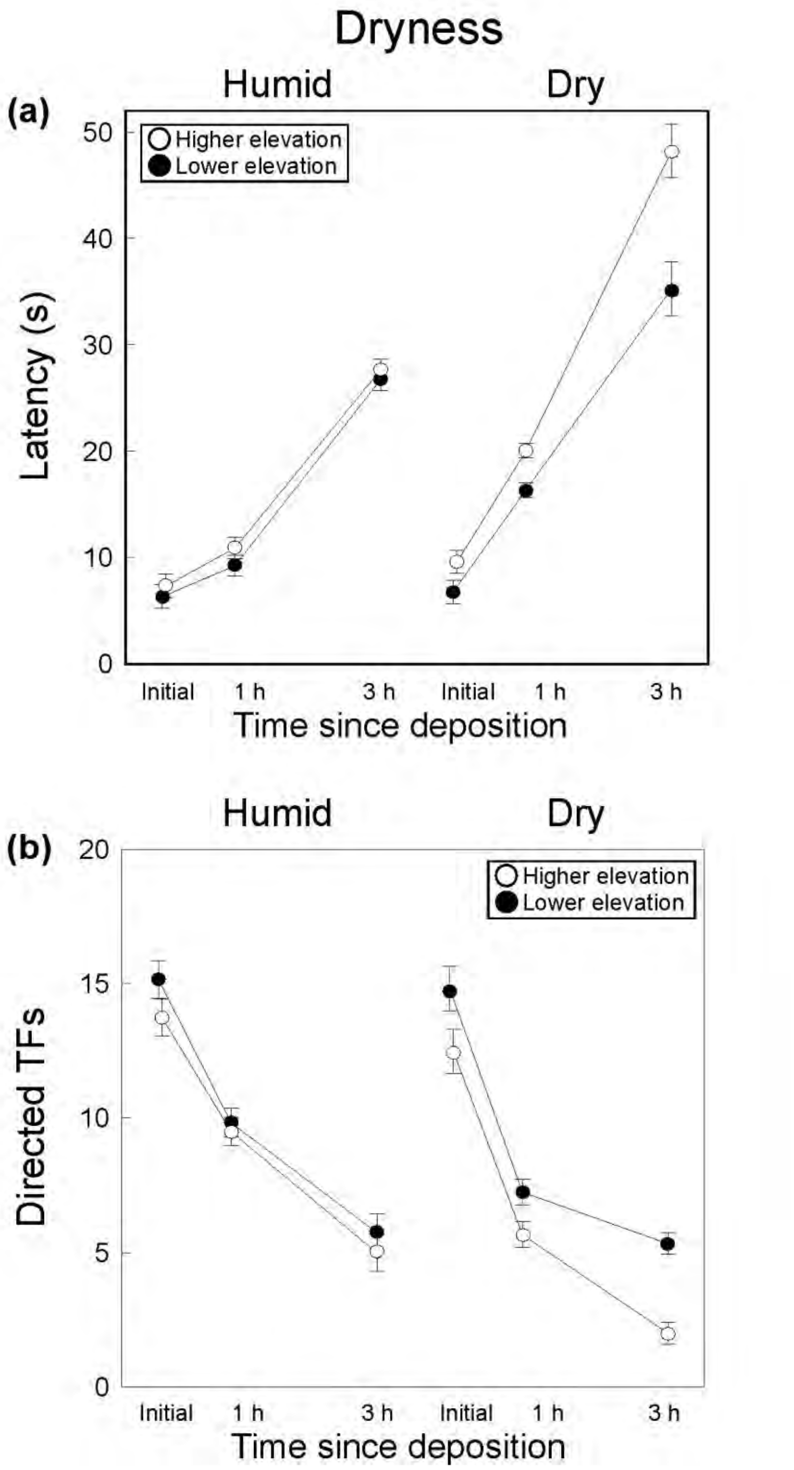

Fig. 3. Effects of Dryness on Signal Efficacy. Mean ± SE (a) latency and (b) number of tongue-flicks (TF) directed to swabs by female P. hispanicus lizards from two populations (lower elevation: black; higher elevation: white) in response to swabs bearing femoral secretions of males immediately after they were secreted ('initial'), 1 h or 3 h since deposition and maintained under two dryness conditions (humid vs. dry).

The number of TFs directed by females differed significantly between populations and dryness treatments, and among time periods, and dryness*population and dryness*time interactions were significant (Table 1; Fig. 3b). Thus, in both populations, TF rates of females decreased as the time since the secretion was deposited increased and when secretions were previously exposed to drier conditions. Therefore, the signal elicited lower responses (i.e. was less effective) as time and dryness increased. However, the significant interactions indicated that the detrimental effect of dryness was more marked in the higher elevation population. Thus, while initial TF rates did not significantly differ between populations nor between dryness treatments (Tukey's tests, $p > 0.07$ in all cases), TF rates 1 and 3 h after deposition did not significantly differ between dryness treatments in the lower elevation population (1h: $p > 0.07$; 3 h: $p = 0.99$) but TF rates were significantly lower in the dryness treatment in the higher elevation population (1h: $p < 0.001$; 3 h: $p = 0.018$) (Fig. 3b).

Discussion

Our results showed that the characteristics of chemical signals of male *P. hispanicus* lizards differed between two populations inhabiting environments with different climatic conditions in spite the fact that these two populations are closely related genetically (Gabirot *et al.,* 2013a). Moreover, chemosensory tests with female lizards showed that chemical signals of males had a lower efficacy (i.e. detectability and persistence) when temperature and dryness increase, but that these effects were more detrimental in the higher elevation population that occupies naturally colder and more humid environments. Therefore, we suggest that interpopulational differences in chemical profiles of femoral secretions of male *P. hispanicus* lizards may reflect adaptation to maximize the efficacy of the chemical signal in different climates. Nevertheless, our study suffers the limitation of considering only two populations, and, to confirm this hypothesis, further studies should include more populations reflecting a richer cline in climatic conditions.

Climatic Conditions and Chemical Signals Composition

Chemical analyses confirmed interpopulational differences in chemical composition of femoral secretions of male *P. hispanicus* lizards, as between other populations [Martín & López, 2006b; Gabirot *et al.,* 2013a, Gabirot *et al.,* 2010). These differences occur not only among genetically distinct populations or species in the *P. hispanicus* species complex (Gabirot *et al.,* 2012b; Gabirot *et al.,* 2010), which might be simply explained by genetic drift without invoking adaptive reasons, but also between populations that are closely related genetically (Gabirot *et al.,* 2013a). The latter suggests that local adaptation to different environments may drive differences in chemical signals.

The results of this and previous studies showed that differences in chemical composition of femoral secretions between these two *P. hispanicus* lizard populations are due to lower elevation males having higher proportions of cholesterol and fatty acids, and lower proportions of alcohols than higher elevation males. These different compositions should result in different physical-chemical properties of the entire femoral secretion under different temperature and humidity conditions. Thus, under the naturally higher temperatures and drier conditions of the lower elevation population, the higher relative proportion of cholesterol (the main compound in secretions) may contribute to avoid degradation of other more easily alterable compounds that are known to be important in intraspecific communication in this lizard such as cholesta-5,7-dien-3-ol (López & Martín, 2005; Gabirot *et al.,* 2012a, Martín & López, 2006), which, however, did not differ between populations. It was already suggested that cholesterol, the major compound in secretions of many lizard species (Weldon *et al.,*

2008) might not have a signalling function, but just form a matrix that could protect other compounds, which would be the true semiochemicals (Escobar *et al.,* 2003).

Female *P. hispanicus* show strong chemosensory responses to both cholesterol and cholesta-5,7-dien-3-ol (but responses are higher to the latter) (Martín & López, 2006a). Females might use the abundant cholesterol to detect, via chemosensory senses alone, the substrate scent marks from males, and later use proportions of the less abundant cholesta-5,7-dien-3-ol to evaluate the quality of the male. Experimental studies indicated that female *P. hispanicus* do not prefer scents marks of males with higher proportions of cholesterol, but prefer marks with higher proportions of cholesta-5,7-dien-3-ol (López & Martín, 2005). This steroid is a precursor of vitamin D_3, which is essential in calcium metabolism and a potent immuno-stimulator (Fraser, 1995). Thus, there could be a potential conflict between allocating high proportions of this steroid to femoral secretions and maintaining simultaneously an appropriate immune response . This trade-off, which only genuinely high quality males may afford (López *et al.,* 2009), would confer honesty to the chemical signal (Martín & López, 2015).

Similarly, the greater abundance of alcohols in secretions of the higher elevation population would not be useful under higher temperatures where alcohols will evaporate more quickly, which may explain why lower elevation males allocated lower proportions of alcohols to secretions. The function of alcohols in secretions is not known, but in other lacertid species, alcohols are related to the social status of a male (i.e. more dominant males have higher proportions of some alcohols in secretions) eliciting in other males aggressive responses (Martín *et al.,* 2007; Khannoon *et al.,* 2011). A previous study showed that male *P. hispanicus* from the lower elevation population showed lower chemosensory responses to alcohols than males from the higher elevation population, suggesting interpopulational differences in relative importance of alcohols in communication (Gabirot et al., 2012c). These differences might be linked to the different efficacy of alcohols in scent marks under different climatic conditions.

Interpopulational differences in chemical composition may be explained not only by local adaptation but also by developmental plasticity as a direct consequence of the environmental thermal conditions experienced by lizards in each population. Thus, an experimental study show that differences in basking conditions in the laboratory cause plastic changes in the composition of femoral secretions of male lizards *Podarcis muralis* (Heathcote *et al.,* 2014). This suggests that total time spent at optimal body temperatures may affect average metabolic rates and general health of lizards, which would result in a modification of their condition-dependent chemical signals (Martín & López, 2015). Similarly, in our study, the observed interpopulational differences in chemical profiles could reflect the long-term effect of having been exposed to their respective native climatic conditions. Nevertheless, in spite of differences in climatic conditions, which do affect directly to deposited scent marks, lizards could be able to obtain similar thermoregulatory precision in both populations through flexible thermoregulatory behaviour (Gabirot *et al.,* 2013b). A further crossed experimental study (population of origin x thermal conditions of scent mark donors) would be necessary to disentangle local adaptation *vs*. developmental plasticity effects. However, both processes would result in a higher efficiency of chemical signals under local climatic conditions.

Effects of Climatic Conditions on Signal Efficacy

With respect to the chemosensory experiments, our results indicate that females detected later (i.e. they had longer latency times) and had lower chemosensory tongue-flick responses to the femoral secretions of males as the time since deposition increased. This indicated that the chemical stimuli in secretions faded with time, very likely because chemical compounds that elicit responses evaporated and degraded with time since they were secreted (Epple *et al.,* 1980)In addition, the loss of detectability and efficacy of the chemical signal was faster under warm temperature and under drier conditions. This is because high temperatures increase evaporation and diffusion rates of chemicals, affecting their persistence (McDonough *et al.,* 1989; Regnier & Goodwin, 1977]. Similar detrimental effects of higher temperatures on the efficacy of scent-marks were found in another lacertid lizard species (Martín & López , 2013)Similarly, high temperatures limit trail-following behaviour of ants by accelerating pheromone decays (van Oudenhove et al., 2011; van Oudenhove et al., 2012).

Interestingly, these detrimental effects of higher temperatures and drier conditions affected differentially secretions of the two populations. Thus, secretions from the lower elevation population, which occupies warmer and drier environments, seem to be less affected by these simulated conditions that resembled local ones. In contrast, secretions from the higher elevation population, which occupies fresher and more humid environments, suffered a quicker degradation of efficiency under climatic conditions similar to those of the lower elevation population. These results support that characteristics of femoral secretions of males are adapted to local conditions of temperature and humidity in order to improve their efficiency. Interpopulational differences in the properties of compounds found in the chemical profiles also support this conclusion. Alternatively, further studies should consider whether the observed differences in chemosensory responses might be explained because the vomeronasal systems of females from each population might also have coevolved to be more efficient in detecting scent marks of males under the local environmental conditions.

In addition to changes in the chemical composition of secretions, the effects of different environmental conditions on the persistence of scent marks might also be compensated by increasing the amount of secretion produced. Mechanisms can be , for example, increasing the number or size of femoral glands in warmer climates to compensate for a quicker evaporation (Escobar *et al.,* 2001; Iraeta et al., 2011). Nevertheless, this strategy alone (i.e. without additionally modifying compounds in secretions), might not be enough to compensate for different climates as interspecific variation in the number of femoral pores in lacertid lizards seemed independent on climatic variables (Baeckens *et al.,* 2011). Also, when unfavourable environmental conditions render chemical signals very costly or not useful, an alternative communication system (i.e. visual) may be favoured (Fox & Shipman, 2003).

Consequences of Local Adaptation of Chemical Signals

What are the consequences of these microgeographic differences in male chemical signals in *P. hispanicus*? If variation in male mating signals to maximize efficacy in local conditions co-evolve with female preferences for the characteristics of the signal of the males of their own population, reproductive isolation and divergence between populations might arise as a consequence (Gabirot *et al.,* 2012a; Gabirot *et al.,* 2012b) This could contribute to explain, at least partially, the genetic divergence observed between populations that inhabit contrasted environments in this lizard species complex (Kaliontzopoulou *et al.,* 2011). Nevertheless, females may use some characteristics, or compounds, of the chemical signal that do not vary between populations in sexual selection (Gabirot *et al.,* 2013a), ignoring other differing compounds. This is because these characteristics may represent the strategic design of the signal, informing honestly on the quality of a male (Martín & López, 2015). Interpopulational differences in chemical signals might only be aimed to protect and maximize the persistence of the compounds that convey the true message, which would be the same in the two populations. Therefore, this might prevent genetic divergence between these populations in spite of differences in chemical signals. Finally, from a conservationist point of view, our study suggests that if climatic conditions (temperature and humidity) change quickly due to global warming, this could decrease the efficacy of chemical sexual signals of wall lizards. This might disrupt sexual selection processes (Martín & López, 2013) affecting survivorship of populations.

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Resumen extenso en español

Determinantes de la variacion fenotípica en el complejo de especies de la lagartija iberica

Introduccion

El estudio de la variación

El estudio de la variación ha fascinado siempre a los biólogos y los intentos por explicar esta fueron decisivos para la formulación de las ideas de Darwin acerca de la evolución (Russell & Bauer, 2005). La ciencia y la biología en particular, le deben mucho a Charles Darwin y Alfred Russel Wallace, por su, aunque sencilla, revolucionaria idea de la evolución por medio de la selección natural. Sin embargo, la influencia de Darwin en el pensamiento moderno va más alla de esta "re-evolución". El darwinismo rechaza las explicaciones y fenomenos sobrenaturales. La teoría de la evolución por medio de la selección natural rompe con la concepción estática del mundo que invoca a dioses como creadores o diseñadores, explica la adaptación y la diversidad de una manera puramente materialista (Mayr, 2000).

El esencialismo, también conocido como tipologia, fue la ideologia predominante en occidente hasta bien entrado el siglo XIX (Mayr, 1982), y tiene sus raices en la filosofia platónica y el refinamiento Aristotélico (Panchen, 1992). La naturaleza variable del mundo era vista como la manifestación imperfecta de las esencia subyacentes, expresadas en la forma y el comportamiento, constituyendo un gran problema para la biología (Panchen, 1992). La variación era considerada como accidental y, por consiguiente, de escaso interés científico (Russell & Bauer, 2005). El esencialismo hace hincapié en la discontinuidad y la estabilidad; validando la realidad de los taxones con independencia de una teoria explicativa (Panchen, 1992) y enlaza directamente con la insistencia de Linneo en la realidad, delimitación precisa y la constancia de las especies (Mayr, 1982).

Darwin superó las limitaciones impuestas por el pensamiento esencialista y consideró la variación en sí misma como una importante fuente de evidencia en contra del esencialismo biológico. Rechazó completamente el pensamiento tipológico e introdujo un concepto totalmete diferente: el pensamiento poblacional (Mayr, 1969). La variación es inherente a cualquier especie y, por tanto, las poblaciones son grupos de individuos diferentes entre sí. Incluso los eres humanos mostramos una extensa variación a nivel morfologico, genético, fisiológico y de coloración (Lewontin, 1972; Serre & Pääbo, 2004; Relethford, 2000; Hanihara, 2008).

La ecología evolutiva es una disciplina relativamente reciente que fusiona la ecologia y la evolucion. De hecho, esta perspectiva histórica ha hecho posible que la ecología evolutiva florezca en una masiva disciplina que ha asimilado y reemplazado a otras subdisciplianas dentro de la ecología (Pianka, 2011). La ecología evolutiva se centra en el estudio de la variación entre individuos, poblaciones y especies. Pero no toda la variación es importante desde el punto de vista evolutivo, solo aquellos caracteres que se transmiten fielmente de generación en generación tienen el potencial de evolucionar. Por lo tanto, que la variación en un carácter sea heredable, es un requisito para que la selección natural actue y produzca adaptaciones. Por ello las causas y los efectos de la variación despiertan un gran interés para los ecólogos evolutivos (Mazer & Damuth, 2001).

Variación en las estrategias vitales

El estudio de las estrategias vitales es una rama de la ecología evolutiva cuyo objetivo es explicar cómo la selección natural y otras fuerzas evolutivas moldean a los organismos para optimizar su supervivencia y su reproducción en respuesta a desafios ambientales (Stearns, 1992; Roff, 1992; Stearns, 2000). Las estrategias vitales más estudiadas son: número, tamaño y sexo de la descendencia; tasas de crecimiento, edad y tamaño en la madurez; inversión reproductiva dependiente del tamaño o la edad; tasas de supervivencia dependientes del tamaño o la edad y longevidad. Estas estrategias vitales son consideradas componentes de la eficacia biológicay su evolución depende de la interaccion entre las fuerzas selectivas y los factores intrinsecos al organismo que afectan a su supervivencia y reproducción (Roff, 1992).

Los trade-offs han jugado un papel central en el desarrollo de la teoríade las estrategias vitales (Stearns, 1989). Se definene como una correlación negativa entre dos estrategias vitales, de manera que el beneficio en la eficacia biológica experimentado al incrementar una de estas es constrarrestado mediante un coste en términos de eficacia biológica al disminuir la otra (Stearns, 1992).

Todas las estrategias vitales deberían evolucionar para maximizar la supervivencia y el éxito reproductivo, incrementando por tanto la eficacia biológica (Houle, 2001). Sin embargo, los recursos son finitos y un organismo no puede invertir al máximo en todos los caracteres La selección no puede maximizar las estrategias vitales más alla de ciertos limites y seleccionará la combinacion de cracteres que maximiza la eficacia biológica. Sin trade-offs y otro tipo de restricciones, la eficacia biológica más alta pertenecería a un “demonio Darwiniano” que se apoderaría del mundo (Law, 1979), un organism hipotético para el que no existirían los trade-offs.

Uno de los ejemplos más destacados de trade-offs en estrategias vitales es el coste de la reproducción. El coste de la reproducción actual tiene dos caras: los costes de supervivencia y los costes de reproducción futura (Stearns, 1989). Las especies que se reproducen a una edad temprana tienen una corta esperanza de vida (raton vs seres humanos) y existe una correlacion negativa entre la fecundidad temprana y tardía (diente de león vs encina) (Stearns, 1992).

La variación en las estrategias vitales no esta solo causada por trade-offs y restricciones. En algunos casos, esta variación es inducida mediante factores ambientales (plasticidad fenotípica) (Losos *et al.,* 2000; Via & Lande, 1985), en otros esta producida por diferencias intrínsecas asociadas a condiciones geográficas variables (adaptación local) (Travis, 1994); o por una combinación de ambos (Sears & Angilletta, 2003). Por tanto, la comprensión de la relevancia ecológica y evolutiva de la variación intraespecica a nivel geográfico precisa de la distinción entre las causas genéticas y ambientales de la variación (Berven *et al.,* 1979, Ferguson & Brockman, 1980; Ballinger, 1983; Berven & Gill, 1983; Jones & Ballinger, 1987; Ford & Siegel, 1989). Por todo ello, las estrategias vitales son muy variables tanto entre especies como entre poblaciones estrechamente emparentadas (Roff, 1992; Stearns, 1992; Du *et al.,* 2005).

El estudio de las estrategias vitales tiene como principal objetivo responder a preguntas como: Cuanto deberia vivir un organismo ? Cuanta descendencia deberia tener y de que tamaño ? Cuantas veces deberia reproducirse ? El estudio de las estrategias vitales consiste en comprender la adaptación, un tema fundamental en biología evolutiva (Fabian & Flatt, 2012).

Comportamiento antidepredatorio

La depredación se define como una interacción en la que un individuo de vida libre mata y obtiene recursos de otro organismo (Peter, 2001) y está considerada como una importante fuerza selectiva en la evolución de adaptaciones (Lima & Dill, 1990; Lima, 1998). La depredación es un proceso que comprende varias etapas por parte del depredador: detección, identificación, aproximación, captura y consumición (Endler, 1991). Los depredadores ejercen una selección más fuerte sobre las presas que la que las presas ejercen sobre los depredadores. El "principio vida-cena" y la asimetria característica de las carreras de armamentos depredador-presa, significan que, en general, las presas tienden a tener la ventaja (Dawkins & Krebs, 1979). Dentro de los caracteres que pueden verse afectados por la depredación, la coloración y el comportamiento han sido estudiadas profusamente. Las White Sands constituyen un buen ejemplo de cómo un cambio evolutivo puede ser inducido por la depredación de una manera muy rapida. Las White Sands son un pintoresco paisaje de dunas de yeso rodeado por los desiertos de adobe oscuro de la cuenca de Tularosa, que crean un ecotono entre los sustratos blancos y negros (Kocurek, 2007). En este lugar, tres especies de lagartijas han evolucionado rápidamente desde una coloración ancestral oscura a una blanquecina: *Holbrookia maculata, Sceloporus undulatus* y *Aspidoscelis inornata*. Esta coloración blanquecina está considerada como una adaptación que facilita la cripsis. De este modo se pone en evidencia como la cripsis es una buena estrategia antidepredatoria que consiste en pasar desapercibido a los depredadores. Sin embargo, la selección mediada por la depredación puede tambien promover la evolución de coloración llamativa si los beneficios al exhibir esta son mayores que los costes (Wilkinson, 2003). Salvo para especies aposemáticas, una coloración llamativa hace más detectable a una potencial presa, de modo que los individuos conspicuos pueden compensar esa mayor susceptibilidad a la depredación con comportamientos antidepredatorios. (Lima & Dill, 1990; Forsman & Appelqvist, 1998). Por ejemplo, las coloraciónes llamativas pueden actuar como señuelos y dirigir los ataques de los depredadores hacia partes no vitales del cuerpo, incrementando con ello las probabilidades de supervivencia (Arnold, 1984; Wilkinson, 2003). Esto es bien conocido en algunas especies de lagartijas que poseen colas vívidamente coloridas en rojo, verde o azul de las cuales pueden desprenderse mediante autotomía (Pianka & Vitt, 2003). La efectividad de una cola conspicua puede verse incrementada mediante movimientos ondulatorios de ésta, incrementando asi las probabilidades de huida (Arnold, 1984; Cooper & Vitt, 1985; Castilla *et al.,* 1999; Watson *et al.*, 2012). No obstante, estas exhibiciones también pueden ser señales disuasorias de una persecución, que advierten a los predadores de que han sido detectados, provocando que estos aborten el ataque (Dial, 1986; Hasson *et al.,* 1989; Cooper, 2007).

Ya que el riesgo de depredación aumenta en cada una de las etapas que van desde la detección hasta la consumición, y puesto que las presas son depredadas por más de una especie de depredador, la selección favorece en las presas aquellas defensas que resultan en una deteccion temprana de los depredadores. Es por esta razón que las presas pueden desarrollar comportamientos antidepredatorios que evitan la detección o a los depredadores (Edmunds, 1974; Lima & Dill, 1990; Endler, 1991). Para evitar a los depredadores, muchos reptiles huyen tan pronto detectan a estos, mientras que otros permiten que se aproxime antes de huir (Vitt & Congdon, 1978; Greene, 1988). Los modelos teóricos, así como la evidencia empírica sugieren que una presa no debería escapar inmediatamente al detectar un depredador que se aproxima, sino que debe ajustar su respuesta de escape para minimizar los costes de la huida ya que un depredador que se acerca no es siempre una amenaza real (Ydenberg & Dill, 1986). Sin embargo, la respuesta general de un saurio al escapar es la huida. Las tácticas de escape varían dependiendo de las características del microhábitat (Snell *et al.,* 1988; Bulova, 1994), condición térmica (Rand, 1964; Hertz *et al.,* 1982; Carrascal *et al.,* 1992), gravidez

(Bauwens & Thoen, 1981; Braña, 1993) o la ontogenia (Pounds *et al.,* 1983). Una huida puede considerarse un éxito si la presa tiene una mayor velocidad y resistencia que el depredador y si es capaz de huir a un refugio donde éste no pueda seguirle (Edmunds, 1974; Endler, 1991). Sin embargo, distintos beneficios energéticos y perceptuales surgen cuando un individuo se desplaza de una manera discontínua (Kramer & McLaughlin, 2001). Este fenómeno se conoce como locomoción intermitente y ocurre en contextos tan diversos como la locomoción, búsqueda o persecución de una presa (Avery *et al.*, 1987). Las paradas breves durante la huida pueden mejorar la vigilancia del depredador así como la percepción del microhábitat y, por tanto, permitir a la presa ajustar su velocidad o la dirección de la huida: Además, las pausas pueden ocasionar la pérdida de contacto con la presa por parte del depredador (Edmunds, 1974; Herzog & Burghardt, 1974) gracias a una coloración críptica que permita a la presa confundirse con el entorno (Martín & López, 1995) o reduciendo la capacidad de este para anticipar la posición de la presa (Driver & Humphries, 1988).

Polimorfismos

Al contrario que los caracteres contínuos, los polimorfismos constituyen excelentes modelos para el estudio de procesos microevolutivos (Iserbyt *et al.,* 2013). Los polimorfismos facilitan y simplifican el estudio de las dinámicas evolutivas en la naturaleza pues son fácilmente catalogados y rastreados (Calsbeek *et al.,* 2010). Una especie es polimórfica cuando en una población de individuos de un mismo sexo y edad aparecen distintas variantes fenotípicas que se heredan genéticamente y para las cuales la expresión no depende ni de la influencia del ambiente ni de la condición física (Roulin, 2004). Podemos encontrar polimorfismos morfológicos, fisiológicos o comportamentales (Galeotti *et al.,* 2003) y a nivel de especie, población u ontogenia (Forsman & Shine, 1995). Entre ellos, los polimorfismos de coloración han sido ampliamente estudiados y son un fenómeno común en muchas especies animales.

Los polimorfismos de coloración son comunes en la mayoría de los vertebrados (Huxley, 1955; Forsman & Shine, 1995; Fuller & Travis, 2004; Roulin, 2004; Hoekstra *et al.,* 2006; Hoffman *et al*., 2006). Los individuos que expresan patrones de coloración diferentes son, en muchos casos, seleccionados para expresar comportamientos alternativos, con diferentes comportamientos o estrategias reproductivas específicamente favorecidas en combinación con cada patrón de coloración (Brodie, 1992; Forsman & Appelqvist, 1998). Por ejemplo, la coloración del plumaje en el cárabo común (*Strix aluco*) covaría con la estrategia reproductiva empleada (Roulin *et al.,* 2003) y los leones de melena más oscura (*Panthera leo*) son más agresivos y sexualmente más activos (West & Packer, 2002). En los lacértidos, se ha estudiado ampliamente la existencia de distintos morfos ventrales que se correlacionan con distintos componentes de la eficacia biológica, como por ejemplo en *P. muralis* y *Z. vivipara*, mientras que al mismo tiempo ha sido completamente pasado por alto en otras especies (e.g. *P. guadarramae*).

Los polimorfismos restringidos a un solo sexo representan excelentes modelos para estudiar la naturaleza de la selección divergente y han sido ampliamente documentados en distintos grupos como insectos, moluscos, isópodos y vertebrados (Oliveira *et al*., 2008). Los estudios de polimorfismos en machos son abundantes, y su mantenimiento tiende a ser explicado por selección dependiente de frecuencia negativa. Esto significa que los fenotipos raros experimentan una ventaja de supervivencia o apareamiento sobre los morfos más comunes (Partridge, 1988; Olendorf *et al.,* 2006). En *Uta stansburiana* los machos naranjas, los cuales son muy agresivos, usurpan el territorio a los machos azules, mientras que estos últimos vencen a los machos amarillos al custodiar a las hembras, y los machos amarillos, a su vez, ganan a los naranjas al copular clandestinamente con sus hembras. Los machos amarillos, con su estrategia “clandestina”, prosperan cuando predominan los machos naranjas territoriales (Sinervo *et al.,* 2001). Sin embargo, las investigaciones recientes demuestran que, a pesar de que existe un sesgo hacia el estudio del polimorfismo en machos, los polimorfismos restringidos al sexo femenino son más abundantes en la naturaleza de lo que se pensaba (Kunte, 2009; Svensson *et al.,* 2009). Cuando solo las hembras son polimórficas en una especie mientras que los machos son monomórficos y no presentan la misma variación que las hembras, hablamos de polimorfismo limitado a hembras (FPP; Stamps & Gon, 1983)

A pesar de que el interés en FPP ha aumentado considerablemente en los últimos años (Svensson *et al.,* 2009; Cox & Calsbeek, 2012; Ortega *et al.,*2014) todavía existe un sesgo hacia su estudio en insectos, en detrimento de los vertebrados (Svensson *et al.,* 2009; Cox & Calsbeek, 2012; Ortega *et al.,*2014). Por ejemplo, FPP se encuentra en diversas especies de caballitos del diablo, libélulas, y mariposas. En muchas de estas especies existen dos o más morfos, uno de los cuales se caracteriza por una coloración o patrón similar a la de los machos (Sirot *et al.,* 2003) el cual se considera como una adaptación para reducir los costes debidos al acoso de los machos en búsqueda de hembras receptivas (Iserbyt *et al.,* 2013).

Aunque gran parte de los estudios se han llevado a cabo sobre especies con polimorfismos de coloración basados en carotenoides o pteridinas (e.g. Sinervo & Zamudio, 2001; Vercken *et al*., 2007; Galeotti *et al*., 2013) existe algún ejemplo de polimorfismos dorsales basados en melaninas. Varias especies de *Anolis* presentan FPP en el que las hembras de una población generalmente muestran dos o tres morfos dorsales (línea vertebral, rombos o escaso patrón como los machos, mientras que los machos suelen tener un patrón escasamente marcado y raramente lineal (Calsbeek *et al.,* 2008; Paemelaere *et al*., 2011) . Además, en *Z. vivipara*, la frecuencia de individuos con un patrón dorsal reticulado es mayor en las hembras que en los machos (Lepetz *et al*., 2009). De manera que parece que existe una tendencia hacia machos no lineales, al menos en algunos machos, lo que sugiere que puedan existir distintas presiones selectivas entre los patrones dorsales de macho y hembras. No obstante, los procesos evolutivos que determinan los polimorfimos restringidos a hembras no están del todo comprendidos. Además, investigaciones recientes han demostrado que existe una relación entre los procesos de especiación y aquellos que mantienen y generan los polimorfismos (Hugall & Stuart-Fox, 2012). Sentados estos precedentes, el estudio de los polimorfismos en general, y de FPP en particular, constituyen una excitante y prometedora área de investigación: Podría ayudarnos a comprender la coexistencia de fenotipos alternativos dentro de una población, lo que constituye uno de los mayores desafíos de la teoría evolutiva.

Señales sexuales

La selección natural puede explicar la evolución de adaptaciones y el proceso de especiación (Darwin, 1859). Sin embargo, muchos caracteres parecen que son una desventaja y maladaptativos, los cuales no pueden haber evolucionado por selección natural porque no parece que aumenten la supervivencia (Andersson, 1994; Møller, 1994). Es más, en muchos casos los individuos aue poseen este tipo de caracteres incurren en un coste de supervivencia al llamar la atencion de los depredadores (Zuk & Kolluru, 1998; Martín & López, 2001). Un buen ejemplo de ello es la viuda de cola larga (*Euplectes progne*). En esta especie las hembras prefieren a aquellos machos con las plumas caudales más largas y, a su vez, una cola larga dificulta el vuelo (Andersson, 1982). Darwin (1871) planteó la hipotesis de que estos caracteres sexuales secundarios extravagantes evolucionan por selección sexual, un proceso por el cual las desventajas en la supervivencia son contrarrestadas por una mejora en las oportunidades de apareamiento debido a las preferencias del sexo contrario. Por lo tanto, la selección sexual trata de la evolución de los caracteres sexuales secundarios que ofrecen a ciertos individuos una ventaja reproductiva sobre el resto. Concretamente, la selección sexual comprende dos mechanismos distintos: la competencia entre machos y la eleccion por parte de las hembras. La siguiente cita de Darwin (1871) lo resume todo a la perfeccion: “Esta batalla entre sexos es de dos clases; una es entre los individuos de un mismo sexo, generalmente los machos, para hacer desaparecer o matar a sus competidores, mientras las hembras permanecen pasivas; en la otra, la lucha es entre individuos del mismo sexo, para excitar o conquistar a los del sexo contrario, las hembras normalmente, las cuales no permanecen pasivas, sino que seleccionan a los pretendientes más apuestos”. Las consecuencas de este dialogo son la evoluciónde señales sexuales que aportan informacion acerca del emisor. Estas señales sexuales pueden afectar a cualquier canal sensorial: vision, tacto, oido u olfato. Sin embargo, llama la atencion el hecho de que el estudio de las señales sexuales esté plagado de ejemplos de brillantes y coloridos caracteres como por ejemplo la coloración nupcial de algunos ciclidos y las aves del paraiso. Esto se debe probablemente al sesgo que tenemos como observadores humanos. Esta atraccion hacia coloraciones llamativas parce ser un subproducto de nuestra propia evolución como primates diurnos.

Señales visuales

En esta categoria se incluyen exhibiciones comportamentales y patrones y coloraciones llamativas. Muchos lagartos diurnos, en su mayoria agámidos e iguánidos, realizan exhibiciones estereotipadas que pueden tener diversas funciones, tales como la identificacion de especies, atraccion de pareja o mediar interacciones agonisticas (Radder *et al,.* 2006). Por ejemplo las lagartijas del género *Anolis* realizan movimientos con la cabeza de arriba a abajo con una frecuencia determinada según la especie (Jenssen, 1977). Sin embargo, estas exhibiciones no forman parte de este trabajo, de modo que nos centraremos en la coloración *per se,* como la de la papada de *Norops sp* la cual maximiza la eficacia de una señal visual ya existente.

En reptiles se han descrito cuatro tipos basicos de células pigmentarias: xantoforos, eritrforos, iridioóforos y melanóforos (Cooper & Greenberg, 1992; Bagnara, 1998). Xantóforos y eritróforos son celulas pigmentarias que absorben la luz y que contienen pteridinas y carotenoides, que dan una coloración que va del amarillo al rojo (Kuriyama *et al.,* 2006). Los iridióforos reflejan la luz gracias a unas plaquetas que contiene purinas y pteridinas, y son responsables de los colores estructurales (Bagnara, 1998). Los melanoforos absorben la mayor parte de la luz, produciendo el color negro y marron. La disposicion de estas células puede producir una amplia gama de colores en la piel de los reptiles (Morrison, 1995; Morrison *et al.,* 1996).

Existen coloraciones que pueden funcionar como señales sexuales que median la comunicacion intra e interespecifica, asi como el reconocimiento entre poblaciones (Rosenblum, 2009). Por ejemplo, los machos de lagarto verde (Lacerta schreiberi y Lacerta viridis) poseen una coloración dorsolateral verde brillante (Václav *et al*,. 2007; Martín & López, 2009). Las caracteristicas de esta coloración, la cual está amenudo basada en carotenoides, puede reflejar el estado de salud de los machos (Ressel & Schall, 1989; Martín *et al.,* 2008; Martín & López, 2009) y puede ser utilizada por las hembras para elegir a los machos.

Señales quimicas

Las señales quimicas son utilizadas por muchos animales en contextos sociales y reproductivos (Wyatt, 2003; Mason & Parker, 2010). La quemorrecepción es por tanto la principal modalidad sensorial empleada por la mayoria de animales, incluyendo a los vertebrados, y las señales quimicas (feromonas o semioquimicos) juegan un papel muy importante tanto en la comunicacion entre sexos y, por tanto, en la selección sexual (Wyatt, 2003; Müller-Schwarze, 2006; Mason & Parker, 2010). En los vertebrados terrestres, las feromonas son comunmente incorporadas en las heces, orina, u otros mecanismos de senalizacion que depositan estas señales en el sustrato con el objetivo de delimitar un territorio o atraer pareja (Lopez *et al.,* 1998; Brennan & Kendrick, 2006).

En saurios, las feromonas son depositadas sobre el sustrato mediante unas estructuras epidérmicas (poros) cercanas a la cloaca. De hecho, la ubicacion ventral de estos poros sugiere que las secreciones se depositan pasivamente sobre el sustrato al desplazarse. Dependiendo de la ubicacion exacta de estos poros hablamos de poros cloacales, precloacales o femorales (Fig). En lacértidos, se encuentranen la cara ventral de los muslos y reciben el nombre de poros femorales. Los poros femorales han sido ampliamente ultilizados en taxonomia, pero se sabe relativamente poco acerca de su funcionalidad (Alberts, 1993). Están formados por una invaginación del estrato germinativo, que forma una unidad folicular y produce una copiosa cantidad de secrecion de manera holocrina (Mason, 1992). Estas estructuras están muy difundidas dentro de squamata, estando presentes en grupos filogenéticamente alejados, como gecos, escincos, agamidos, iguanidos, lacértidos y teidos

Objetivos

El principal objetivo de esta tesis es el studio de los factores que determinan la variación fenotípica en el complejo de especies de la lagartija ibérica (*Podarcis hispanicus*). Para alcanzar esta meta, la tesis se divide en seis capitulos, cada uno con los siguientes objetivos :

Capitulo I. Determinar las causas de las diferencias morfológicas y comparar la inversión reproductiva en dos poblaciones de *P. guadarramae* distribuidas a lo largo de un gradiente altitudinal con una contrastada climatología. Concretamente, queremos determinar el papel de la adaptación local frente a la plasticidad fenotípica.

Capitulo II. Examinar la influencia de las condiciones ambientales en el crecimiento d elas crias de las dos poblaciones mencionadas en el capitulo anterior. También medimos la disponibilidad de alimento en ambos sitios para esclarecer la relación existente entre la productividad del ecosistema y el crecimiento.

Capitulo III. Determinar el valor adaptativo de la conspicua coloración azul de la cola y de los distintos morfos de coloración dorsal, así como su relación con el comportamiento de escape en *P. guadarramae.* De la misma manera examinamos la influencia de la población de origen sobre la coloración de la cola y el comportamiento de escape.

Capitulo IV. Contrastar la hipótesis en la que la selección actua de manera diferencial sobre los distintos morfos dorsales en hembras, examinando distintos índices de fitness. También comparamos las diferencias en el crecimiento y la morfología de las crías descendientes de ambos morfos.

Capitulo V. Comparar las diferencias fenotípicas entre poblaciones de elevada y baja altitud de la lagartija parda *P. liolepis*, perteneciente a este complejo de especies. Queremos averiguar si el patrón de variación encontrado en *P. guadarramae,* en el centro peninsular, se repite en esta especie.

Capitulo VI. Examinar la eficacia (persistencia) de las secreciones femorales a diferentes temperaturas y niveles de humedad para comprobar si las señales químicas están adaptadas a las condiciones ambientales locales.

Aportaciones fundamentales de esta tesis

Variación altitudinal en el fenotipo de los adultos y en las estrategias vitales

Las lagartijas de altitud elevada tuvieron un tamaño corporal mayor así como cabezas más robustas y fémures más largos. El **Capítulo I** ofrece una clara evidencia de que el divergente tamaño corporal entre poblaciones de elevada y baja altitud no es consecuencia del tamaño al nacer, el cual no está afectado por el tamaño del huevo, ni las tasas de crecimiento intrínsecas de los neonatos, en relación con las condiciones ambientales experimentadas en nuestro experimento "common garden". Las hembras de *P. guadarramae* de la población de elevada altitude realizaron puestas de mayor tamaño, más pesadas y más voluminosas, y estas diferencias fueron independientes de su mayor tamaño corporal. Este resultado podría indicar que la inversión reproductiva pueda estar determinada por las condiciones ambientales, las cuales, a su vez, afectarían a la productividad del ecosistema.

El resultado más significativo del **Capítulo I** es la ausencia de diferencias en las tasas de crecimiento de las crías bajo condiciones estandarizadas en el laboratorio (experimento common garden). Debido a ello, las diferencias morfológicas presentas en los adultos no son consecuencia de diferencias intrínsecas (genéticas) sino que se deben a una respuesta plástica a los factores ambientales durante el desarrollo.

En el **Capítulo II,** mediante un diseño experimental de tranplante recíproco, averiguamos que las crías de *P. guadarramae* crecieron más rápido a elevada altitud, con independencia de la población de origen. La disponibilidad y la diversidad de alimento fueron mayores a elevada altitud, lo que se correlaciona con el mayor crecimiento de las crías. Este hallazgo confirma que las condiciones ambientales y sus efectos sobre la productividad primaria juegan un papel crucial en las diferencias morfológicas de los adultos de elevada y baja altitud.

Valor adaptativo de la coloración conspicua de la cola y de los morfos dorsales

El **Capítulo III** demuestra que el comportamiento antidepredatorio de las crías de *P. guadarramae* se ve influenciado por la interacción entre el patrón dorsal, el tamaño y la coloración conspicua de la cola. No hallamos diferencias entre sexos o morfos en relación a la composición del espectro visible de la cola. Sin embargo, las crías hembra reticulada-manchadas tienen una reflectancia mayor en el rango ultravioleta (UV) del espectro, que las crías hembra rayadas y los machos reticulados-manchados, mientras que las hembras rayadas tiene un nivel de reflectancia UV intermedio y los machos reticulado-manchados tuvieron los niveles más bajos.

Estas marcadas diferencias entre machos y hembras reticulado-manchados en reflectancia UV, contrastan con sus similitudes en el comportamiento de escape, ya que tienen una mayor velocidad media y máxima, y pasan un menor tiempo paradas que las hembras rayadas. Esto sugiere una asociación entre el morfo dorsal y determinados aspectos del comportamiento de escape independientes del sexo. Aunque no medimos específicamente la resistencia durante la carrera, podemos interpretar que la estrategia de escape de las hembras rayadas podría basarse en una mayor resistencia, debido a que una menor velocidad máxima y media permitiría recorrer mayores distancias. Por el contrario, tanto hembras como machos reticulados-manchados recurrirían a una estrategia basada en recorrer huir hacia el refugio más próximo en el menor tiempo posible.

En el **Capítulo III** se pone de manifiesto como las crías con una coloracón de la cola más llamativa, es decir, más brillante, adoptan un comportamiento más tímido, parando menos durante la huida, reduciendo así el riesgo de depredación debido a su mayor detectabilidad por parte de los depredadores.

Por otro lado, no encontramos diferencias entre poblaciones en la coloración de la cola. Sin embargo los machos de la población de baja altitud tendieron a realizar más movimientos ondulatorios de la cola que los de elevada altitud. Además, cuanto menor fue el tamaño corporal de éstos, mayor era el número de movimientos ondulatorios de la cola, lo que hace pensar que estos movimientos son una estrategia antidepredatoria llevada a cabo por aquellos individuos extenuados tras la huida, como último recurso.

Los resultados del **Capítulo IV** muestran como los morfos dorsales de las hembras de *P. guadarramae* difieren en cuanto a su morfología adulta, coloración basada en melaninas e inversión reproductiva. Las hembras rayadas, independientemente de su edad, tienen un mayor porcentaje de melaninas (coloración negra) y un menor porcentaje de coloración marrón que las reticuladas-manchadas, mientras que no difieren en el porcentaje de coloración amarillenta. Además, las crías tienen un mayor porcentaje de melaninas, y un menor porcentaje de coloración marrón. Este hecho puede estar relacionado con las dificultades termorregulatorias impuestas por un pequeño tamaño corporal (baja inercia térmica, rápidas tasas de calentamiento y de enfriamiento). Las hembras reticuladas-manchadas tienen cabezas más altas y fémures más largos que las hembras rayadas, lo que las hace morfológicamente más similares a los machos. Esto podría a puntar a una base fisiológica común independiente del sexo en los individuos reticulados-manchados.

Los morfos dorsales de *P. guadarramae* tienen estrategias reproductivas diferentes. Las crias cuyas madres son reticuladas-manchadas son más grandes y pesadas que aquellas cuyas madres son rayadas. Esto puede deber bien a efectos maternos o a diferencias genéticas entre los morfos. No obstante, este hecho, unido a la mayor similitud morfológica entre individuoes reticulados-manchados, hace pensar que diferencias fisiológicas entre los morfos son las responsables de este patrón.

No encontramos diferencias en cuanto a tamaño de puestas y huevos, tasas de crecimiento o respuesta inmune entre las hembras de diferentes morfos. De manera similar, el morfo de la madre no influenció la tasa de crecimiento de las crías, como tampoco lo hizo el porcentaje de melaninas. Finalmente, el porcentaje de melaninas no influenció las tasas de crecimiento de las crías.

Variación altitudinal en P. liolepis

En el **Capítulo V** vemos como las *P. liolepis* de elevada altitud son más grandes y robustas que las de baja altitud, siguiendo la regla de Bergmann. Este resultado es exactamente el mismo que el encontrado en poblaciones de *P. guadarramae* del centro peninsular. Esto nos hace pensar que la regla de Bergmann puede ser el patrón general en este complejo de species, al contrario que en la mayoría de saurios. Otro aspecto en común entre *P. liolepis y P. guadarramae* es el mayor número de poros femorales a elevada que a baja altitud.

En primavera, las *P. liolepis* de baja altitud tienen un mayor brillo de la coloración ventral que las de elevada altitud, mientras que en verano sucede lo contrario. Además la reflectancia de radiación azul de la zona gular es mayor en verano que en primavera. La reflectancia de radiación azul también es mayor en machos en todas las regiones corporales salvo en la zona gular. La coloración roja es más intensa en la zona gular, decreciendo paulatinamente hacia el vientre y la zona precloacal.

En cuanto a la coloración dorsal, las *P. liolepis* de baja altitud tienen un mayor brillo de la coloración ventral que las de elevada altitud, mientras que en verano sucede lo contrario. Las lagartijas de baja altitud son también más verdes dorsalmente que las de elevada altitud. De manera similar, la coloración dorsal de las lagartijas fue más verdosa y menos marrón que en verano.

Eficacia de las señales químicas

El **Capítulos VI** confirma las diferencias poblacionales descritas previamente en estas dos poblaciones de *P. guadarramae*. Las secreciones femorales de los machos de baja altitud tienen mayores proporciones de colesterol y de ácidos grasós, así como una menor proporción de alcoholes que los machos de elevada altitud.

Los experimentos de quemorrecepción realizados en este capítulo indican que las hembras tienen mayores tiempos de latencia y realizan menor cantidad de protrusiones linguales a las secreciones d elos machos a medida que pasa el tiempo tras la deposición de las secreciones. Este resultado se debe, probablemente, a que los compuestos que desencadenan la respuesta se evaporan y degradan desde su deposición. Además, esta pérdida de detectabilidad y eficacia de las señales químicas fue más rápida bajo condiciones de alta temperatura y baja humeda, como consecuencia de una mayor tasa de evaporación y de difusión de los compuestos químicos, que afecta a su persistencia. Esta pérdida de eficacia en condiciones de alta temperatura y baja humedad es más acusada en las secreciones de los machos de elevada altitud, los cuales habitan en un ambiente más húmedo y fresco. Tales resultados encajan con que las secreciones femorales estás adaptadas a las características ambientales para mejorar su eficiencia.

Conclusiones

- El experimento "common garden" demuestra que el divergente tamalo corporal de *P. hispanicus* en dos poblaciones distribuidas a diferente altitud no es consecuencia del tamaño corporal al nacer, el cual no depende del tamaño del huevo, ni de las tasas de crecimiento de los neonatos asociadas con las condiciones ambientales del experimento. Este hecho pone de manifiesto que las diferencias fenotípicas a nivel morfológico no son el resultado de diferencias intrínsecas, sino las consecuencia de una respuesta plástica durante el desarrollo.

- El trasplante recíproco pone de manifiesto que el ambiente en el que se produce el crecimiento determina las tasas de crecimiento, con independencia de la población de origen, en dos poblaciones de *P. hispanicus* estrechamente emparentadas a lo largo de un gradiente altitudianl de 500 m con condiciones ambientales contrastadas. Los factores determinantes de la tasa de crecimiento son probablemente las diferencias de disponibilidad y abundancia de alimento (artrópodos) entre localidades.

- En general, demostramos la existencia de dimorfismo sexual y entre morfos en la reflectancia ultravioleta de las colas de las crías de *P. hispanicus.* Las crías hembras reticuladas-manchadas tienen una mayor reflectancia ultravioleta en sus colas que las hembras rayadas y los machos reticulados-manchados, mientras que las hembras rayadas tienen valores intermedios y los machos muestran los valores más bajos. Además, identificamos el sexo, patrón dorsal, tamaño corporal y brillo de la cola como predictores de distintos aspectos del comportamiento de escape de las crías , y dos estrategia de escape alternativas entre las crías reticuladas-manchadas y las rayadas que probablemente tienen que ver con el patrón dorsal, con independencia del sexo. Las hembras reticuladas-manchadas y los machos (todos ellos reticulados-manchados), corren más rápido y permanecen parados menos tiempo durante las pausas que las hembras rayadas, lo que apunta a una estrategia basada en la resistencia en estas últimas. Las crías más grandes corrieron distancias más largas y realizaron menos movimientos ondulatorios de la cola que las crías de menor tamaño. Por otro lado, las crías macho de baja altitud hicieron movimientos ondulatorios como un último recurso, lo que sugiere que esta estrategia antidepredatoria podría estar relacionada con la fatiga. Además, las crías con colas más brillantes realizan menos pausas entre sprints que aquellas con colas menos llamativas, adoptando un comportamiento más tímido que minimiza su riesgo de depredación durante la huida. Por lo tanto, el comportamiento antidepredatorio de las crías está influido por la interacción entre el patrón dorsal, el tamaño y la coloración conspicua de la cola.

- Los dos morfos dorsales en hembras de *P. hispanicus* tienen estrategias reproductivas diferentes. Las crías descendientes de hembras reticuladas-manchadas fueron de mayor tamaño y más pesadas que las descendientes de hembras rayadas, lo que sugiere que efectos maternos son responsables de estas diferencias fenotípicas de las crías entre los morfos. Los morfos dorsales en las hembras y clases de edad difieren en base a la coloración marrón y la basada en melaninas. Las crías y hembras adultas rayadas son más oscuras (tienen más melanina) que las crías y hembras reticuladas-manchadas. Las hembras adultas de diferente morfo dorsal son morfológicamente diferentes, las hembras reticuladas-manchadas tienen cabezas más altas y fémures más largos que las hembras rayadas. Nuestro resultados sugieren que este polimorfismo dorsal restringido a hembras no está mantenido por presiones electivas relacionadas con la respuesta inmune. De igual modo, negamos la existencia de una relación entre la coloración basada en melaninas, el crecimiento de las crías y la respuesta inmune en hembras adultas. Finalmente, la respuesta inmune no difiere entre los morfos dorsales de hembras adultas.

- Las *P. liolepis* de elevada altitud son más grandes, más robustas, tienen más poros femorales y una coloración dorsal más oscura que las de baja altitud. Las *P. liolepis* de alta y bala altitud siguen el mismo patrón de variación que el observado en otras especies dentro del complejo de especies de *P. hispanicus.* La coloración dorsal y ventral fluctúan entre estaciones y poblaciones. El brillo de la coloración dorsal y ventral es mayor a baja que a elevada altitud en primavera, mientras que en verano encontramos la relación inversa. Además, la reflectancia azul de la zona gular es mayor en verano que en primavera y también es mayor en todas las regiones corporales de los machos salvo en la zona gular. Determinamos la existencia de un gradiente antero-posterior de coloración roja, con los valores más altos en la zona gular. Además, la coloración marrón es más alta en verano que en primavera, mientras que la coloración verde es mayor en primavera que en verano.

- Confirmamos la existencia de diferencias en la composición química de las secreciones femorales de dos poblaciones de *P. hispanicus* estrechamente emparentadas a lo largo de un gradiente altitudianl de 500 m con condiciones ambientales contrastadas. Los machos de baja altitud tienen una mayor proporción de colesterol y ácidos grasos, pero menor proporción de alcoholes en sus secreciones femorales que los machos de elevada atitud. Los test quemosensoriales con hembras muestran que las señales químicas de los machos tienen una menor eficacia a medida que pasa el tiempo tras su deposición, la temperatura y la sequedad ambiental aumentan, pero estos efectos son más acusados en la población de elevada altitud, la cual habita bajo condiciones de mayor humedad y menor temperatura. Por tanto, las diferencias interpoblacionales en los perfiles químicos de las secreciones femorales de los machos de *P. hispanicus* quizá reflejen una adaptación para maximizar la eficacia de las señales químicas bajo distintas condiciones climáticas.

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Abstract

The study of natural variation has long fascinated evolutionary biologists and attempts to account for it were major contributors to the formulations of Darwin´s idea of evolution. The main goal of this thesis is to study the factors that shape phenotypic variation in the Iberian wall lizard species complex (*P. hispanicus)*. We specifically focused on two recently described species within this species complex, *P. guadarramae* and *P. liolepis*. We first performed a common garden experiment (**Chapter I**) to examine differences in reproductive investment and the underlying basis of the altitudinally divergent phenotypes of two *P. guadarramae* populations located along an altitudinal gradient with contrasted climatic conditions. We also performed a reciprocal transplant experiment (**Chapter II**) aiming to determine the influence of proximate effects of local environmental conditions on hatchling growth in lizards from the two populations commented above. We also measured food availability in both sites to determine the relationship between ecosystem productivity and growth. In **Chapter III**, our main goal was to disentangle the factors that shape variation in conspicuous colourful tails and dorsal pattern morphs and its relationship with escape behaviour in *P. guadarramae* juvenile lizards. In **Chapter IV**, we tested the hypothesis that selection acts differentially among *P. hispanicus* female morphs (reticulated-blotched vs. striped) to create alternative morph-specific phenotypic optima at different levels, by testing whether morphs differ in several fitness proxies. For this, we measured morphology, dorsal coloration, reproductive investment and immune response of adult female morphs, and morphology, growth, and dorsal coloration of their offspring. In **Chapter V** we examined altitudinal variation in morphology, and ventral and dorsal coloration populations in adult *P. liolepis*. In **Chapter VI**, we examined the efficacy (i.e., persistence and detectability) of sexual chemical signals of males (i.e. femoral gland secretions) at different temperatures and

humidity levels to test the hypothesis that chemical signals of male lizards have evolved to maximize efficacy of chemical signals in different environmental conditions (highland vs lowland).

The common garden experiment (Chapter I) shows that altitudinally divergent adult body sizes of *P. hispanicus* lizards are not driven by size at hatching, which is not contributed to by egg size, nor intrinsic post-hatching growth rates associated with the environmental conditions experienced in the experiment. Thus, adult phenotypic differences are the result of a plastic response which was latter confirmed by a reciprocal transplant experiment (Chapter II) where the growing environment determines growth rates, independently of population of origin. The drivers of growth rate differences are likely between-sites differences in food availability and quality.

We also provide evidence for the existence of sexual and intermorph dimorphism in tail ultraviolet colour reflectance of hatchling *P. hispanicus* lizards (Chapter III). Hatchling reticulated-blotched females have more UV reflectance in their tails than striped females and reticulated-blotched males, while striped females have intermediate UV reflectance and males the lowest UV reflectance. In addition, we identify sex/dorsal morph, body size and brightness as predictors of different aspects of escape behaviour, and two alternative escape strategies between striped and reticulated-blotched hatchlings that are probably dependent on dorsal morph differences, independently of sex. Reticulated-blotched individuals run faster and spend less time paused than striped females, which might reflect an escape behaviour strategy based on endurance in striped females. Larger hatchlings run longer distances and tail wave less than smaller hatchlings. In addition, lowland males display tail waving as a ‘last resort’ antipredator strategy that may be related to fatigue. Moreover, hatchlings with brighter tails take fewer pauses between sprint bursts than those with duller tails, adopting a shier behaviour that may minimize predation risk during flight episodes. Hence, hatchling antipredatory behaviour is influenced by the interactions between dorsal patterns, size and tail conspicuousness.

The two melanin-based dorsal morphs of female *P. hispanicus* have contrasted reproductive strategies (Chapter IV). Hatchlings born from reticulated-blotched females are larger and heavier than those born from striped ones, which suggest the role of maternal effects mediating offspring phenotype differences between the two morphs. Female dorsal morphs and age classes differ in terms of melanin based, and brownish dorsal coloration. Adult striped and hatchling females are darker than reticulated-blotched adult females and hatchlings, respectively. Moreover, adult female morphs have different morphology as reticulated-blotched females have 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 adult immune response. Finally, immune response do not differ between adult female dorsal morphs.

Highland *P. liolepis* lizards are larger, stockier and have more femoral pores and a darker dorsal coloration than lowland ones (Chapter V). Thus, highland and lowland *P. liolepis* follow the same pattern of variation observed in other species within the *P. hispanicus* species complex. Dorsal and ventral coloration fluctuate seasonally and between populations. Brightness of ventral and dorsal coloration are higher in lowland than in highland lizards in spring whereas the reversed trend is found in summer. In addition, lizards in summer had more throat bluish reflectance than in spring and is also males had more bluish reflectance for all body regions except for the throat. We determine the existence of an anterior-posterior gradient of red ventral coloration, with the highest values in the throat. In addition, summer lizards had more brownish coloration than spring ones whereas spring lizards had more greenish coloration than summer ones.

Finally, the chemical secretions of male *P. hispanicus* lizards differ between two populations (Chapter VI). Lower elevation males have higher proportions of cholesterol and fatty acids, but lower proportions of alcohols than higher elevation males. Moreover, chemosensory tests with female lizards show that chemical signals of males have a lower efficacy when time since deposition, temperature and dryness increase. These effects are more detrimental in the higher elevation population that occupies naturally colder and more humid environments. Therefore, interpopulational differences in chemical profiles of femoral secretions of male *P. hispanicus* lizards might reflect adaptation to maximize the efficacy of the chemical signal in different climates.

Resumen

En esta tesis estudiamos los factores responsables de la variación fenotípica en el complejo de especies de la lagartija ibérica (*P. hispanicus)*. Nos centramos en dos especies, *P. guadarramae* y *P. liolepis*. En el **Capítulo I**, realizamos un experimento "common garden" para comparar la inversión reproductiva y comprender las causas de las diferencias fenotípicas de dos poblaciones de *P. guadarramae* a lo largo de un gradiente altitudinal con contrastadas condiciones climáticas. También realizamos un trasplante recíproco en el **Capítulo II** para determinar la influencia de las condiciones ambientales sobre el crecimiento de las crías. De manera adicional registramos la disponibilidad de alimento para establecer la relación existente entre la productividad del ecosistema y el crecimiento. En el **Capítulo III** medimos con un espectrofotómetro la coloración de la cola de las crías y registramos su comportamiento de escape para averiguar qué variables afectan a la conspicua coloración de la cola, los patrones dorsales y su relación con el comportamiento de escape en *P. guadarramae.* En el **Capítulo IV** medimos la morfología, coloración dorsal, inversión reproductiva y respuesta inmune en hembras adultas reticuladas-manchadas y rayadas. Además, medimos la morfología, coloración dorsal y crecimiento de las crías descendiente ambos morfos dorsales. Examinamos si los morfos difieren en distintos índices de eficacia biológica. En el **Capítulo V** examinamos la variación altitudinal en la morfología, coloración dorsal y ventral en *P. liolepis*. En el **Capítulo VI** determinamos la eficacia de las señales químicas de las secreciones femorales de los machos bajo diferentes condiciones de temperatura y humedad para ver si están adaptadas a las condiciones ambientales de cada población.

El experimento “common garden” (Capítulo I) demuestra que el divergente tamaño corporal de *P. hispanicus* no es consecuencia del tamaño corporal al nacer, el cual no depende del tamaño del huevo, ni de las tasas de crecimiento neonatales asociadas con las condiciones ambientales del experimento sino el resultado de una respuesta plástica durante el desarrollo, hecho que confirma el trasplante recíproco. El ambiente en el que crecen las crías determina las tasas de crecimiento, con independencia de la población de origen. La tasa de crecimiento es probablemente consecuencia de las diferencias de disponibilidad y calidad de alimento entre localidades.

Las crías hembra reticuladas-manchadas tienen una mayor reflectancia ultravioleta en sus colas que las hembras rayadas y los machos reticulados-manchados, mientras que las hembras rayadas tienen valores intermedios y los machos muestran los valores más bajos (Capítulo II). Además, identificamos el sexo, patrón dorsal, tamaño corporal y brillo de la cola como predictores del comportamiento de escape de las crías , y dos estrategias de escape alternativas entre morfos que probablemente tienen que ver con el patrón dorsal, con independencia del sexo. Las crías reticuladas-manchadas, corren más rápido y permanecen parados menos tiempo durante las pausas que las hembras rayadas, lo que apunta a una estrategia basada en la resistencia en estas últimas. El tamaño corporal se correlaciona positivamente con la distancia recorrida, y negativamente con el número de movimientos ondulatorios. Por otro lado, las crías macho de baja altitud hicieron movimientos ondulatorios como un último recurso, lo que sugiere que esta estrategia antidepredatoria podría estar relacionada con la fatiga. Además, las crías con colas más brillantes realizan menos pausas entre sprints que aquellas con colas menos llamativas, adoptando un comportamiento más tímido que minimiza su riesgo de depredación durante la huida.

Los dos morfos dorsales en hembras de *P. hispanicus* tienen estrategias reproductivas diferentes (Capítulo IV). Las crías descendientes de hembras reticuladas-manchadas fueron de mayor tamaño y más pesadas que las descendientes de hembras rayadas. Esto sugiere que efectos maternos son responsables de estas diferencias fenotípicas de las crías entre los morfos. Los morfos y clases de edad difieren en base a la coloración marrón y melaninas. Las crías y hembras adultas rayadas son más oscuras que las crías y hembras reticuladas-manchadas. Las hembras adultas reticuladas-manchadas tienen cabezas más altas y fémures más largos que las rayadas. Parece que este polimorfismo dorsal restringido a hembras no está mantenido por presiones electivas relacionadas con la respuesta inmune. También negamos la existencia de una relación entre la coloración basada en melaninas, el crecimiento de las crías y la respuesta inmune en hembras adultas. La respuesta inmune no difiere entre hembras adultas de distinto morfo.

Las *P. liolepis* de elevada altitud son más grandes, más robustas, tienen más poros femorales y una coloración dorsal más oscura que las de baja altitud (Capítulo V). Las *P. liolepis* de alta y bala altitud siguen el mismo patrón de variación que el observado en otras especies dentro del complejo de especies de *P. hispanicus.* La coloración dorsal y ventral fluctúan entre estaciones y poblaciones. El brillo de la coloración dorsal y ventral es mayor a baja que a elevada altitud en primavera, mientras que en verano encontramos la relación inversa. Además, la reflectancia azul de la zona gular es mayor en verano que en primavera y también es mayor en todas las regiones corporales de los machos salvo en la zona gular. Determinamos la existencia de un gradiente antero-posterior de coloración roja, con los valores más altos en la zona gular. Además, la coloración marrón es más alta en verano que en primavera, mientras que la coloración verde es mayor en primavera que en verano.

La composición química de las secreciones femorales difiere en dos poblaciones de *P. hispanicus* (Capítulo VI). Los machos de baja altitud tienen una mayor proporción de colesterol y ácidos grasos, pero menor proporción de alcoholes que los machos de elevada atitud. Los test quemosensoriales con hembras muestran que las señales químicas de los machos tienen una menor eficacia a medida que el tiempo tras su deposición, la temperatura y la sequedad ambiental aumentan. Estos efectos son más acusados en la población de elevada altitud, la cual habita bajo condiciones de mayor humedad y menor temperatura. Por tanto, las diferencias interpoblacionales en las secreciones femorales de *P. hispanicus* quizá reflejen una adaptación para maximizar la eficacia de las señales químicas bajo distintas condiciones climáticas.