Crypsis in the lizard *Psammodromus algirus* along an altitudinal gradient in Sierra Nevada (Granada, Spain)

Cripsis en la lagartija *Psammodromus algirus* en un gradiente altitudinal en Sierra Nevada (Granada, España)

Trabajo de Fin de Máster
Máster en Conservación, Gestión y Restauración de la Biodiversidad

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ABSTRACT.- *Psammodromus algirus'* dorsal colouration varies along an altitudinal gradient in Sierra Nevada (Spain). An observed parallel variation in ground colour suggests this variation could be due to crypsis. To examine this possibility, we sampled the colouration of the lizards and environmental elements in populations at different elevations along the gradient. Then, we analysed the relation between lizards' colouration and the colour of their environment as well as their relation to lizards' biological features. Results evidenced crypsis being in play and varying along the gradient. Inter-population differences in the degree of crypsis appear to be determined by differences among populations in predation pressure or in relative importance of other selective pressures over colouration (thermoregulation and UV-protection). On the other hand, intra-population variance could be the result of the presence of mixed strategies in response to predators (crypsis or fleeing), associated to differences in behaviour and metabolic resource allocation according to the lizard condition (tail regeneration and female gravidity).

RESUMEN.- La coloración dorsal de *Psammodromus algirus* varía a lo largo de un gradiente altitudinal en Sierra Nevada (España). La variación paralela observada en la coloración del sustrato sugiere que esta variación podría deberse a cripsis. Para examinar esta posibilidad, se muestreó la coloración de las lagartijas y su medio en poblaciones a distintas altitudes a lo largo del gradiente. Tras esto, analizamos las relaciones entre la coloración de las lagartijas y el color de su medio y examinamos su vínculo con las características biológicas de las lagartijas. Los resultados evidencian que existe cripsis, pero en grado variable a lo largo del gradiente. Las diferencias interpoblacionales en el grado de cripsis parecen estar determinadas por diferencias en la presión de depredación o en la importancia relativa de otras presiones selectivas sobre la coloración (termorregulación y protección-UV) entre poblaciones. Por otro lado, la variación intrapoblacional podría ser el resultado de la presencia de estrategias mixtas (cripsis y huida), principalmente asociadas a diferencias en comportamiento o partición de los recursos metabólicos según la condición de las lagartijas (regeneración de la cola y gravidez en las hembras).

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1.- INTRODUCTION

1.1.- Environmental and phenotypic gradients

Gradients are widespread within natural ecosystems as a result of environmental conditions varying progressively. In a gradient, natural populations of a species inhabit the area with a range of conditions in which they can survive and reproduce, in other words, their “fundamental ecological niche”. Frequently this area is where competitors are weaker or absent. If competitors are present and strong, the potential ecological niche is reduced to the species’ “realized ecological niche”. Namely, the area within the ecological niche of a species in which it is able to prevail over its competitors (Hutchinson 1957; Chase & Leibold 2003).

Across the species’ realized ecological niche, environmental conditions vary. At optimal environmental conditions, probability of presence and reproductive success of the species are maximized, being lower elsewhere. Within the optimal range of conditions, as probability of presence is maximized, intraspecific competition can be strong leading to increased selective pressures. In suboptimal ranges, selection can be strong also due to environmental selective pressures. In both cases, if new or already present genotypes in the population have a selective advantage over the rest, the establishment of new characters or character states (phenotype) is favoured over time, generating local adaptation (Dobzhansky et al. 1977; Merrel 1981). Thus, differential genetic flux between populations and differential selective pressures over an environmental gradient can generate a parallel phenotypic gradient (Martin et al. 2010).

An example of this differential phenotype distribution is Gloger’s rule, noted by Gloger (1833). It states that birds’ feathers tend to be darkly coloured in habitats where relative humidity is high and pale where it is low. At least in song sparrows (*Melospiza melodia*), the cause for this variation is the fact that dark feathers are more resistant to bacterial degradation, a severe problem in humid habitats where bacteria thrive, and a lesser problem in arid habitats (Burtt & Ichida 2004).

Alternatively, in some cases, selection pressures do not lead to a particular favoured phenotype but to phenotypic plasticity, which allows individuals to acclimatize to the environment they inhabit. Phenotypic plasticity usually appears as a result of physiological responses to the present conditions triggering different development pathways. Developmental, physiological, and reproductive plasticity have been extensively described, especially in plants (Bradshaw 1965; Travis 1994; Schmitt et al. 1999; Sultan 2000; and references therein). Also, recent studies have documented developmental, physiological and behavioural plasticity in amphibians, reptiles, birds, marine and freshwater invertebrates, insects, mammals, and even lichen (West-Eberhard 2003; Pigliucci 2001; DeWitt & Scheiner 2004). Altricial young birds’ wing development rate in relation to nest ectoparasite infections is an example of phenotypic plasticity. In heavily infected nests, young birds’ wings grow at a faster rate than in non-infected nests. Faster wing growth has been attributed to be a response to ectoparasite infections, which allows young birds to leave the nest earlier (Szép & Møller 2000). Lower weight at the time of nest departure seems to be compensated by reduced exposure time to ectoparasites. Due to the fact that phenotypic plasticity can generate geographical phenotypic differences, it can also lead to parallel phenotypic and environmental gradients.

These phenomena have been frequently reported in many species and habitats. Specifically, much attention has been paid to latitudinal gradients (i.e. Robberecht et al. 1980; Oleksyn et al. 1998; Laugen et al. 2003; Ulstrup et al. 2006; Ostonen et al. 2007). For example, Bergmann's rule predicts larger body size in endotherms in high latitudes, where temperatures are low (Bergmann 1847). This can also be applied to altitudinal temperature gradients (Zamora-Camacho et al. 2012a).

These types of parallel gradients can provide important insights into how species adapt to varying conditions. This is of great importance for understanding ecosystem dynamics, both at global and local scale, especially in a Global Change context. Research at global scale often implies high costs and/or efforts which render them non-viable. In these cases local scale studies have proven to be an extremely useful tool. They allow learning about local ecosystem dynamics while obtaining valuable information which can be used to focus global scale research efforts.

1.1.1.- Altitudinal gradients

Altitudinal gradients share several characteristics with latitudinal gradients (Stevens 1992; Körner 2007; Graae et al. 2012) but can take place within shorter distances (Körner 2007). Thereby, they can be considered as field laboratories which enable us to learn about the dynamics and properties of ecosystems at local scale while gaining insight in to what could be happening at a global scale (Stevens 1992). However, we must be cautious with this approach, as not all environmental factors change in the same manner in altitude as in latitude. Ultraviolet (UV) radiation, for example, increases with altitude but decreases with latitude (Pahkala et al. 2002).

The abiotic and biotic factors that mainly exert pressure over organisms and usually vary with altitude are: temperature (Grant & Duhnam 1990; Körner 2007; which specially affects ectotherms), UV-radiation intensity (Blumthaler et al. 1997; Belden et al. 2000; Sola et al. 2008), cover and type of vegetation (Whittaker & Niering 1975; Woldu et al. 1989; Grytnes & Vetaas 2002) and predation pressure (Menge & Sutherland 1987; Boyle 2008). In response to these clines, animals present different adaptations. For example, many vertebrates follow Bergmans' rule (Ashton et al. 2000; Ashton 2002a, b), presenting larger body sizes with increasing altitude or latitude as an adaptation to low temperatures, although in some clades the tendency is inverted

(Ashton & Feldman 2003). Female Hawaiian damselflies (*Megalagrion calliphya*) also vary in colouration with altitude, presenting two morphs: green and red. Red females dominate at elevated exposed areas whereas green ones dominate in low shaded areas. Red colouration is correlated with superior anti-oxidant ability that might protect from UV damage caused by solar radiation in exposed areas (Cooper 2010). Also, several studies have demonstrated differences in crypsis and survival among colour morphs of different populations in snakes (e.g. King 1992).

1.1.2.- Colouration gradients

The pigmentation system is a particularly promising character to explore connections between genotype and phenotype for ecologically important traits (Hoekstra 2006). Nevertheless, the study of colouration gradients has been specially challenged as often colouration is a result of multiple selective pressures and thus, involves a high degree of complexity. Colouration is relevant for many aspects of organisms' capability to reproduce and survive. It is often used in intra and interspecific communication. Conspicuous colourations are usually associated with social exhibitions (Verbeek 1972; Cooper & Greenberg 1992; Díaz 1993; López et al. 2004). While as non-conspicuous (cryptic) colourations are useful for avoiding detection by predators or preys by mimicking the environment's colouration or patterns (Sheppard 1951; Kettlewell 1955; Camin & Ehrlich 1958; review in Caro 2005). Nevertheless, colouration can also evolve in response to physical factors such as temperature or UV-radiation (Porter & Norris 1969; Jong et al. 1996; Clusella-Trullas et al. 2007; Karl et al. 2009), inconspicuousness being incidental.

When trying to explain non-conspicuous colouration differences among populations, much attention has been paid to melanisation. Melanin is known to cause variation in skin darkness within and across species (True 2003) and its biology is highly conserved across vertebrates (Bennett & Lamoreux 2003). There are two types of melanic pigments (Ito & Wakamatsu 2003): eumelanin (black to brown colourations), and pheomelanin (red-yellow colours). In melanocytes, several genes are involved in the coordination of 'pigment type-switching' between the synthesis of eumelanin and pheomelanin (Barsh 1996). Despite some authors have indicated reptiles lack pheomelanin (Ito & Wakamatsu 2003), recent studies have provided proof this is not true at least for some species (Roulin, Mafli & Wakamatsu 2013).

Both genetic and environmental sources may impact on the degree of melanisation, yet the former appears to be more widespread than the latter (Ellers & Boggs 2004; Espeland et al. 2007; Lee et al. 2008). Variation in pigmentation is often assumed to result from adaptive evolution. Melanisation is involved, for example, in cryptic colouration (Gvozdik 1999; Lorioux et al. 2008), aposematic signalling and mate recognition (Holloway et al. 1995; Kapan 2001; Ellers & Boggs 2003), protection from UV-radiation damage (Gunn 1998), disease resistance (Wilson et al. 2001), and thermoregulation (Clusella-Trullas et al. 2007). Reptiles, and especially lizards, are closely affected by all these evolutionary drivers.

Nevertheless, when evaluating drivers such as crypsis or aposematic signalling and mate recognition, considering only the effect of melanisation (melanic or not melanic morphs) might result insufficient. In such cases, a more holistic approach when measuring colouration should be considered, such as spectrophotometer measurements or photography (Montgomerie 2006).

1.2.- Dorsal colouration in reptiles

Reptiles' ectotherm condition makes them spend long periods of time basking (warming), exposing to UV-radiation damage and predator attacks. Moreover, in this group, conspicuous mating colourations are frequent, especially in males (Carretero 2002; Salvador 2011; Pérez i de Lanuza 2012) further elevating predation risk (Martín & López 1999). Colour pattern and body colouration could be interpreted as a trade-off between different selective pressures including crypsis, intraspecific interactions (Carretero 2002) and environmental constraints. Ventral and lateral zones are more variable in colouration, less conspicuous for predators and directly involved in social exhibitions in comparison to dorsal colouration (Pérez i de Lanuza 2012). In this study, we focused in the latter, which can be selected by factors such as thermoregulation, UV protection or crypsis.

1.2.1.- Thermoregulation hypothesis

In lizards, the effect of dorsal colouration on thermoregulation can be especially relevant within an altitudinal gradient. Melanism (understood as skins' darkness) can enhance an organism's ability to warm up, as stated by "the thermal melanism hypothesis" (Jong et al. 1996; review in Clusella-Trullas et al. 2007; Karl et al. 2009). Dark pigments in the skin can rise body temperature by absorbing a high fraction of solar radiation acting as heat retainers. Thus, individuals with dark colourations (abundant dark pigments) will heat up faster at a given level of solar radiation when behaving equally (Watt 1969; Clusella-Trullas et al. 2007, 2008), giving them an advantage in cold environments. In warm environments, the advantage can shift to light coloured individuals, as the opposite condition could involve overheating problems. Also, in environments where intense melanism does not pose an advantage it is usually reduced by natural selection. Melanism involves substantial costs (Talloen et al. 2004), such as the allocation of amino-acid precursors (phenylalanine, tyrosine) or the regulation of specific enzymes within the biosynthetic pathway (True 2003). When variations in morph frequencies are examined (i.e. Kjaergaard 1981; Gibson & Falls 1988; Terhivuo 1990; Forsman 1995), darker morphs are usually found in high altitude or coastal and island populations. This suggests that thermoregulation through melanism is an important trait in areas with low ambient temperatures and high solar radiation (Gibson & Falls 1988).

1.2.2.- Protection against UV-radiation damage hypothesis

Also, as stated by the "protection against UV-radiation damage hypothesis" (Porter & Norris 1969), and due to the fact that melanin can absorb UV-radiation (Zellmer 1995; Hessen 1996; Gunn 1998), a darker skin (which supposedly possesses a higher concentration of melanins) can act as a protection against cellular damage caused by it. As stated before, UV-radiation increases with altitude (as the thickness of the atmosphere is reduced), but decreases with increasing latitude (as the angle with which the sun incises decreases).

1.2.3.- Crypsis hypothesis

Lastly, dorsal colour variation could also be a result of selective pressures exerted by visual-oriented predators, natural selection favouring cryptic individuals (Endler 1978, 1980; Andersson 1994; Deutch 1997; Zuk & Kolloru 1998; Caro 2005). Crypsis is a widespread mechanism in vertebrates and has extensively been described, especially in mice. In studies concerning the latter, dorsal pigmentation and substrate colour were measured and compared, and the adaptive significance of substrate-matching was tested. Dice & Blossom (1937) described the nearly perfect match between pocket mice's (genus *Chaetodipus* and *Perognathus*) dorsal colouration and substrate colouration, which ranged from nearly black basaltic lava to brilliant white gypsum dunes, along a 25-Km-long transect in Tularosa Basin (New México). Around the same time, Sumner (1929a, b) documented the extremely pale phenotypes of mice in comparison to their darker inland counterparts in the sandy dunes of Florida's Gulf and Atlantic coasts. However, variation in these beach mice (*Peromyscus spp.*) was mainly found in patterning.

Adaptive substrate matching along altitudinal gradients is not limited to mammals. For example, phenotypic plasticity in the brook trout (*Salvenilus fontinalis*) allows it to match background (substrate) colouration during development (Donelly & Whoriskey Jr 1991; Williams 2007). This way, predation risk by hooded mergansers (*Lophodytes cucullatus*) is reduced. In rivers with substrate colour variation along their course, a cryptic colouration gradient is generated as a consequence.

This kind of variation has also been found in reptiles, including lizards (Norris & Lowe 1964), and selective drivers have been elucidated (Luke 1989). For example, Vidal et al. (2007) suggest that crypsis is responsible for the variation of colour patterns in *Liolaemus tenius* (Squamata, Liolaeminae) in Chile. They observed a tendency towards colour matching with the predominant colour of the environment (i.e. predominance of dark colours towards scrublands).

Care should be taken when considering crypsis as the result of selective pressure over dorsal colour variation in altitude. We could predict that, if predation risk varies within a gradient, the importance of crypsis will also vary across it. When the constraint of predation over the animals' colouration is reduced, other drivers, such as mating (which often favours conspicuous colourations) may arise or gain intensity. For instance, Thorpe & Brown (1989) examined the variation in colour pattern found between lizard *Gallotia galloti* populations of the north and south face of the island of Tenerife. Environmental conditions in the north face (less solar radiation and a persistent cloud cover) force individuals to spend more time in the open in order to thermoregulate, increasing predation risk by visual predators as a result. As a consequence, conspicuousness was restricted by predation pressure in northern face populations. On a later study, Brown & Thorpe (1991) found the same phenomena in the skink *Chalcides sexlineatus*, and added a new hypothesis involving variation in vegetation cover. In scarcely vegetated areas, detection by predators is much easier despite crypsis (Brown & Thorpe 1991), rendering the cryptic strategy ineffective. As a result, in southern populations skinks have a preference for an escape strategy and show non-conspicuous tail colourations which are used to focus predator attacks (the tail can be autotomized).

1.3- Dorsal colour variation of Psammodromus algirus *along the altitudinal gradient of Sierra Nevada*

In Sierra Nevada (south-eastern Spain), variance in dorsal colouration with altitude has been detected along lizard *Psammodromus algirus'* (Large Psammodromus) populations (Reguera et al. 2014). Darker lizards can be found at high altitudes (over 2000 metres above sea level, m. asl; Reguera et al. 2014). Previous studies have attempted to determine the selective pressures behind this gradient: "thermal melanism hypothesis" (Zamora-Camacho et al. 2012a) and "protection against UV-damage hypothesis" (Reguera et al. in preparation). However, the "cryptic colouration hypothesis" (another non-exclusive hypothesis which may explain this variation in colouration) has not yet been considered. Ground colour also darkens at higher altitudes (personal observation). This suggests lizards' dorsal colouration gradient could be the result of a cryptic strategy, favoured by the selective pressure exerted by visual avian predators.

Furthermore *P. algirus* is frequently targeted by visual avian predators (Salvador 2011), the predation rate of which are known to be strongly affected by the degree in which their prey match substrate colouration (Dice 1947; Kaufman 1974). This consideration has often been taken into account in other studies, such as the ones carried out to analyse pupal melanisation in insects (Wiklund & Sillentullberg 1985; Ferreira et al. 2006; Jones et al. 2007). The importance of crypsis could vary according to sexual condition in this lizard. Males are much more active than females during the breading season due to courtship and territorial defence, activities which imply a decreased survivorship due to predation (Díaz 1993; Salvador et al. 1996).

2.- OBJECTIVES

We propose a set of questions in order to determine the relationship between the lizards' dorsal colouration and the environmental elements' colouration along the altitudinal gradient:

1. - How does environmental elements' colouration vary with altitude?

2. - How does lizards' dorsal colouration vary with altitude?

3. - Is there any relation between the variation in environmental colouration and lizards' dorsal colouration?

4. - How does the difference between lizards' dorsal colouration and the colour of their environment vary in altitude?

5. - How are lizards' colouration and its difference with environmental elements' colouration related to lizards' biological features?

3.- MATERIALS AND METHODS

3.1.- Species description

Psammodromus algirus is a lacertid 60-80 mm snout-vent length (SVL), abundant in shrubby Mediterranean habitats. It extends from south-eastern France to Morocco, including most of the Iberian Peninsula, along a wide elevation range (0-2600 m. asl), where it is exposed to diverse environmental conditions (Salvador 2011; figure 1).

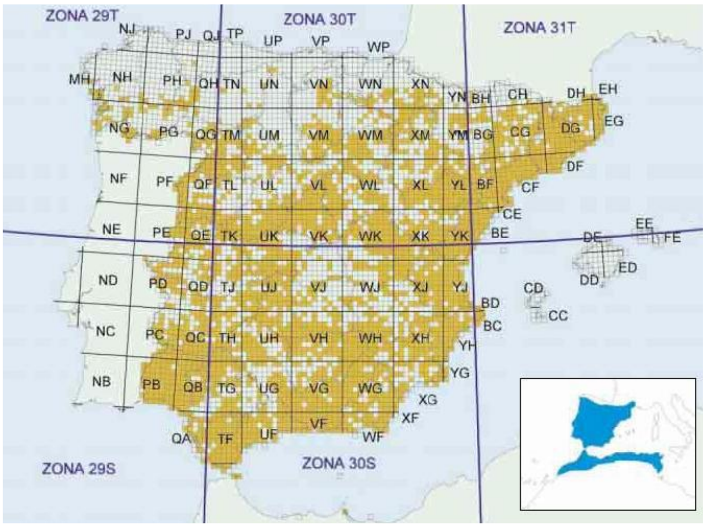

Figure 1. – Map of *Psammodromus algirus*' distribution in the Iberian Peninsula. Taken from Carretero et al. (2002).

Dorsal ground-colouration goes from pale to dark brown, with two dorsum-lateral yellow stripes and a third mid-vertebral stripe in eastern populations (Salvador 2011; Verdú-Ricoy 2013). Ventral ground-colouration goes from white to grey, frequently with yellow pigmentation in the throat region that varies in extension and intensity (Carretero 2002). In western populations, males show orange pigmentation around the throat region during the breeding season (Salvador 2011), but not in eastern populations, where males only show an orange spot in the commissures (Carretero 2002), as can be noticed in figure 2.

Regardless *P. algirus* has been assigned to the Gallotiinae subfamily within the family Lacertidae, its taxonomical position remains unclear (Verdú-Ricoy et al. 2010). Carranza et al. (2006) proposed that Iberian populations were divided into two different lineages, western and eastern, about 3.6 million years ago. Either way, populations examined in this study all belong to the same clade (eastern lineage). In both lineages, tail and limb lengths as well as head width are typically greater in males when compared to females. Also, males have more numerous and bigger lateral ocelli (2-7 in males, 1-3 in females) and femoral pores (Salvador 2011). Females have longer body trunks associated with their egg-storing capacity (Olsson et al. 2002).

Figure 2. - Occidental (A) and oriental (B) Iberian populations’ model male (left) and female (right). All images from Salvador (2011).

Due to its ectothermic nature, the body temperature of *P. algirus* depends on environmental temperature and radiation with an optimum at 31.4ºC (Carretero & Llorente 1995; Díaz 1997). The species depends on a heliothermic strategy for thermoregulation associated with ethological patterns (Díaz & Cabezas-Díaz 2004). Generally, it basks at ground level always within proximity of bushes or shrubs. Habitat vegetation is an important factor for this species’ biology (Díaz et al. 2005). Díaz (1992) observed this lizard selects maximum solar incidence periods within the day for basking. This behaviour enhances their thermoregulatory efficiency. Shorter exposure times are required for lowering predation risk. Nevertheless, some days in summer, when temperatures are extreme, central hours of the day are avoided (personal observation).

The abundance of *P. algirus* is highly correlated with shrub and bush vegetation cover (>50cm in height) as well as with food availability (Díaz & Carrascal 1991), which in turn is also correlated with near ground and dead vegetation, as well as the understory of all Mediterranean forests, sclerophyll or conifer based (Carretero 2002).

Psammodromus algirus is an opportunistic predator that feeds on species with large population numbers. In this way, resource limitation is avoided, optimizing energy intake (Carretero & Llorente 1993) and probably reducing the time invested in aliment acquisition (Díaz & Carrascal 1993). The great majority of consumed species are included within four orders: Hymenoptera, Hemiptera, Orthoptera and Araneae (Díaz & Carrascal 1990). It usually follows an active foraging strategy, employing the vomeronasal system as well as sight and audition to detect preys within vegetation (dry and fresh) or on the ground. However, it is also known to use a “sit and wait” strategy (Salvador 2011).

Active foraging strategy is conditioned by prey availability. When preys are abundant (beginning of spring) the number of preys consumed is high and their size, small. As prey abundance decreases, less number of preys are consumed and bigger sizes are selected. This variation in strategy seeks to maximize time and energetic efficiency and minimize predation risk (Díaz 1993).

Conservation status:

- World scale: Least Concern (LC) (IUCN, Mateo-Miras et al. 2009).
- National scale (Spain): Least Concern (LC) (Carretero et al. 2002).

3.2.- Description of the study area

The study area was located in the south face of Sierra Nevada, province of Granada, in the southeast of Spain (2º56'1‟2 - 3º38'02‟2 of west longitude and 36º56'10‟0 - 37º12'58‟9 of north latitude). Sierra Nevada Mountain belongs to the Penibetic Mountainous System. It extends over 2,000 Km^2, and run from east to west for nearly ninety Km. Sierra Nevada constitutes the southernmost mountain system in Europe and reaches 3,481 m. asl at its highest point, Mulhacén peak. The Penibetic System was originated when the African and Euro-Asiatic tectonic plates met crushing the Alborán plate in between and forcing it to wrinkle. As a result, materials were elevated reaching the highest altitudinal range in the Iberian Peninsula.

Annual mean precipitations vary from 267 to 753 mm (data obtained from REDIAM, Red de Información Ambiental de la Consejería de Medio Ambiente de la Junta de Andalucía, http://www.juntadeandalucía.es/medioambiente/site/rediam). In winter and above 2,000 m. asl precipitation usually falls as snow, and covers the ground around six months each year, from November to May. Below two thousand metres above sea level precipitations usually take place during spring and autumn in the form of rain. Along with ice melt waters during summer, precipitations keep the ground humid all year long, despite the dry summer season typical of Mediterranean climates (Valle 2004). Mean annual temperatures range from 17.6 to 3.5ºC from the lowest to the highest point of the mountain chain (data obtained from REDIAM; figure 3). Relative irradiance varies with altitude on average 6-8% per kilometre for UV-A radiation and 7-11% per kilometre for UV-B (Sola et al. 2008). From a bioclimatic perspective, the territory presents from thermo-Mediterranean to oro-Mediterranean thermotype and from dry to sub-humid ombrotype.

Ground characteristics vary along the altitudinal gradient. At lower altitudes, vegetation is sclerophyll and has more gaps. Due to this, erosive processes are more intense and lead to shallow clear coloured floors with low accumulations of organic matter. On the contrary, at higher altitudes organic matter is better retained giving the ground a darker colouration. Ground colour could also be caused by the floors original material. If so, light colouration (low plots;

figure 4) would be caused by quartzite dominating over dark mica-schist. These materials correspond to the Nevadense-Filabrico complex, in which all plots are included. Vegetation presents in the study area is included in the Nevadense-Filabrico and Alpujarrense districts, which belong to the Nevadense and Alpujarrense-Gadorense sectors in the Baetic province (Valle 2004).

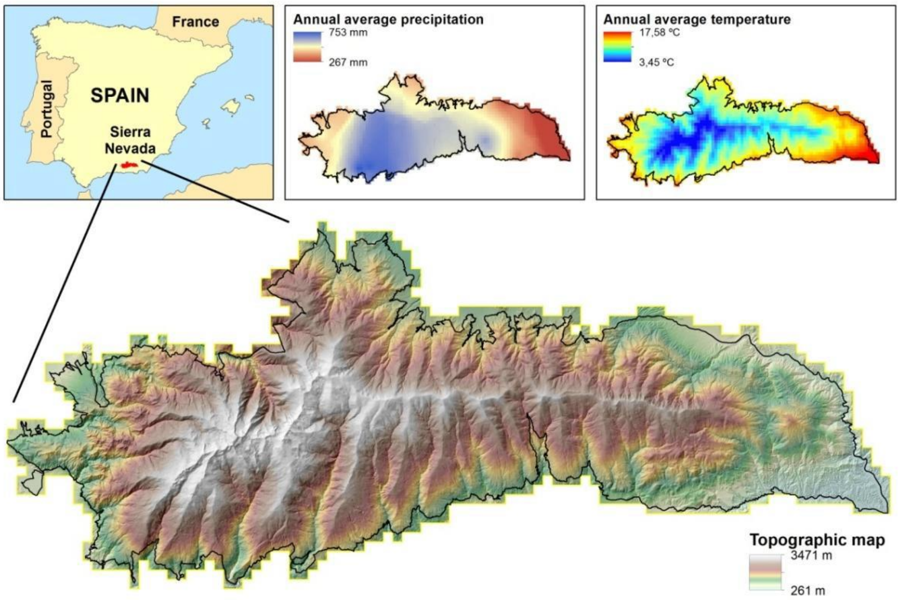

Figure 3. – Geographical location of Sierra Nevada within the Iberian Peninsula (top left), mean annual precipitation range (top centre), mean annual temperature range (top right), and topographical range (bottom) of Sierra Nevada. Elaborated with data obtained from REDIAM, Red de Información Ambiental de la Consejería de Medio Ambiente de la Junta de Andalucía (http://www.juntadeandalucía.es/medioambiente/site/rediam).

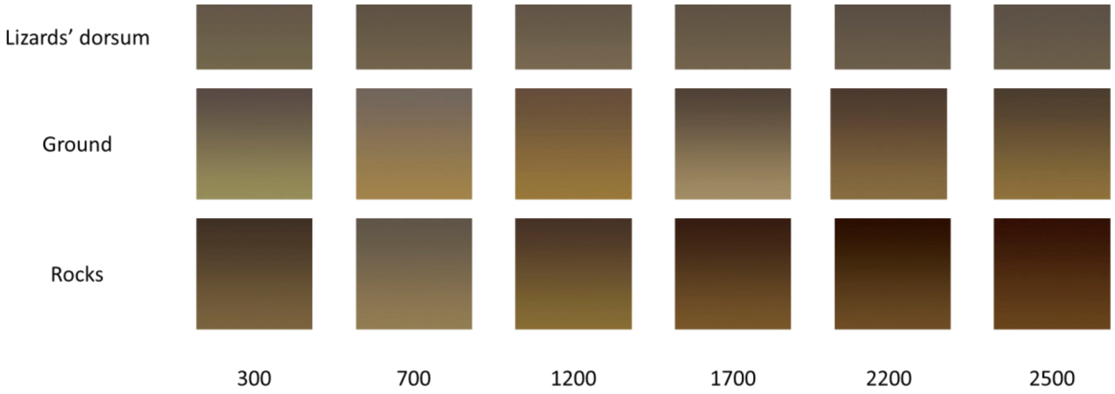

Figure 4. – Variance in colour along the altitudinal gradient for *P. algirus'* dorsum and its main basking sites (ground and rock).

3.3.- Sampling plot selection

Sampling was always carried out within the habitat typically selected by *P. algirus*: areas with abundant bushes and shrubs ranging from 40 to 100 centimetres high and relatively high vegetation cover (Díaz & Carrascal 1991). Care was taken into selecting plots with similar habitat structure although plant communities varied significantly along the gradient, which in turn spans through three different bioclimatic regions (thermo-, suprameso- and oro-Mediterranean).

Six sampling plots were established along the altitudinal gradient separated approximately 500 m of altitude: 300, 700, 1200, 1700, 2200 and 2500 m. asl (figure 5). All plots were within the altitudinal range of *P. algirus'* distribution, from 0 to 2600 m. asl (Fernández-Cardenete et al. 2000). Sampling plots were distributed within two contiguous valleys. Sampling plots from 300 to 1700 m. asl were located in the valley formed by Seco River while sampling plots at 2200 and 2500 were in the valley of Poqueira River.

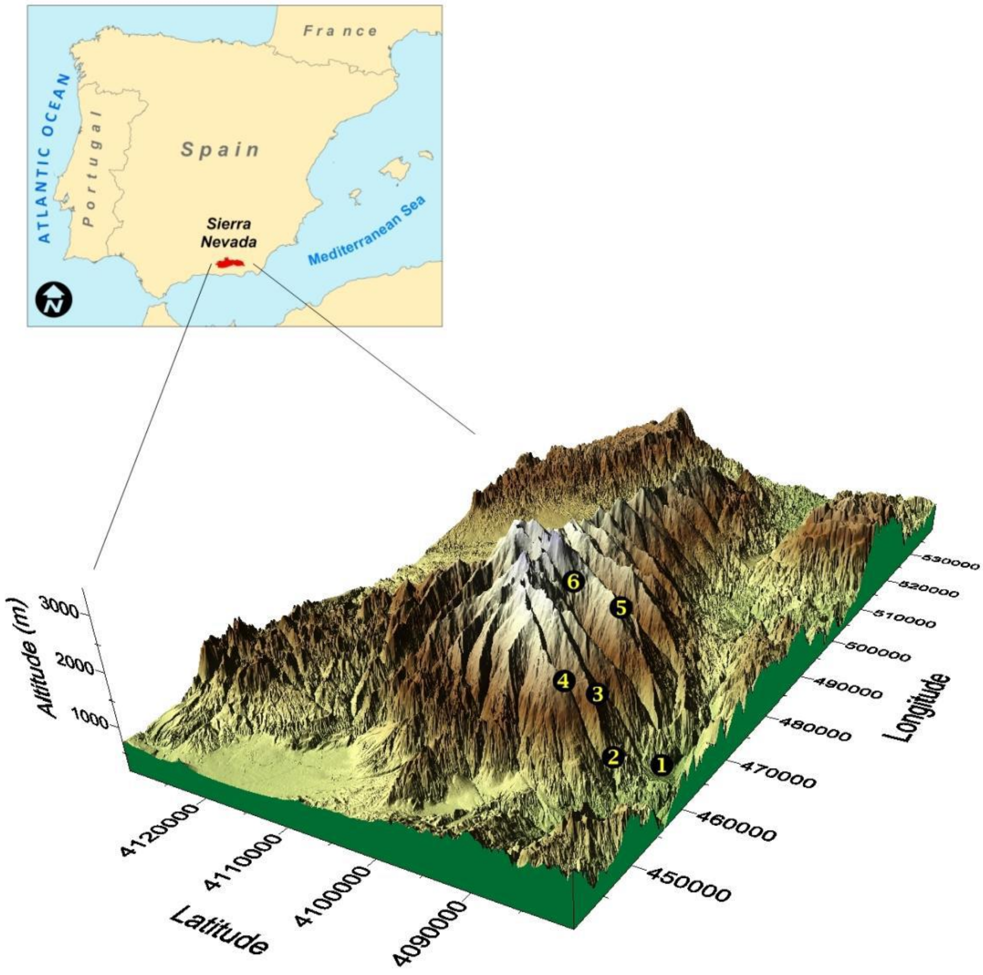

Figure 5. – Three-dimensional representation of the six sampling plots (1 = 300 m. asl, 2 = 700 m. asl, 3 = 1200 m. asl, 4 = 1700 m. asl, 5 = 2200 m. asl and 6 = 2500 m. asl). Elaborated with data obtained from REDIAM, Red de Información Ambiental de la Consejería de Medio Ambiente de la Junta de Andalucía (http://www.juntadeandalucía.es/medioambiente/site/rediam).

3.3.1.- Plot 300

300 m. asl (36°53' N, 3°24' W). The climate is characterized by warm summers and frost-free winters. The potential plant community corresponds to the thermo-Mediterranean series of *Quercus rotundifolia*. Nevertheless, due to human alteration only shrubs and bushes typical of these series, such as *Rosmarinus officinalis*, *Retama sphaerocarpa*, *Genista* sp., *Artemisia sp*. or *Thymus sp*., can be found (Valle 2004). Also, *Eucalyptus sp*. and fruit-trees have been introduced and are present. In non-forest areas, shrubs and bushes cover around 53% of the ground with a mean height of 17.86 cm (Zamora-Camacho et al. 2013).

3.3.2.- Plot 700

700 m. asl (36° 55'N, 3°26'W). Climatology is similar to that on plot 300 due to the influence of the Guadalfeo River which maintains thermo-Mediterranean conditions. Similarly to plot 300, only shrubs and bushes of the thermo-Mediterranean series of *Quercus rotundifolia* can be found, along with *Olea europea*, *Prunus dulcis* and *Ficus carica* plantations. Notwithstanding, the presence of an asphalted road is the most significant human alteration in this plot. In non-forest areas, shrubs and bushes covered around 67% of the ground with a mean height of 23.21 cm (Zamora-Camacho et al. 2013).

3.3.3.- Plot 1200

1200 m. asl (36°56'N, 3°25'W). The climate is characterized by warm summers and frequent frosts in winter. Potential plant community corresponds to the suprameso-Mediterranean series of *Quercus rotundifolia* with *Retama sphaerocarpa*. The bushes and scrubs of the series previously described were present (Valle 2004) and essential for our study. In non-forest areas, shrubs and bushes covered around 54% of the ground with a mean height of 11.75 cm (Zamora-Camacho et al. 2013).

3.3.4.- Plot 1700

1700 m. asl (36°57'N, 3°26'W). The climate is characterized by warm summers and frequent frosts in winter, during which the ground is covered with snow. Potential plant community corresponds to the nevadensic suprameso-Mediterranean series of *Quercus pyrenaica*, accompanied with vegetation correspondent to the suprameso-Mediterranean series of *Quercus rotundifolia* in the areas that most exposed to the sun. Again, bushes and scrubs of the first described series were present (Valle 2004). In non-forest areas, shrubs and bushes covered around 66% of the ground with a mean height of 20.54 cm (Zamora-Camacho et al. 2013).

3.3.5.- Plot 2200

2200 m. asl (36°58'N, 3°19'W). The climate is characterized by warm summers and very frequent frosts and snowfall in winter, during which the ground is covered with a considerably thick layer of snow, variable in depth. Potential plant community corresponds to the oro-Mediterranean series of *Junniperus communis subsp*. *nana*. Also, of vital importance to our study was the presence of a dominant shrub, *Genista versicolor*, in the clearings formed within the *Pinus sylvestris subsp*. *nevadensis* forest cultivations that are widespread in this area (Valle 2004). In non-forest areas, shrubs and bushes covered around 82% of the ground with a mean height of 4.17 cm (Zamora-Camacho et al. 2013).

3.3.6.- Plot 2500

2500 m. asl (37°01'N, 3°19'W), around ice-melt water springs and in the vicinity of Mulhacén River. Climatology is similar to that on plot 2200 with even more extreme conditions during winter. Potential plant community corresponds to the oro-Mediterranean series of *Junniperus communis subsp. nana* (Valle 2004). In this plot (lacking forested areas), shrubs and bushes cover around 66% of the ground with a mean height of 8.93 cm (Zamora-Camacho et al. 2013).

3.4.- Sampling

Due to *P. algirus*' ectothermic nature, the following factors were considered when planning the sampling calendar and schedule: mid-high ambient temperatures, low or null possibility of precipitations and/or wind, and avoidance of central hours of the day during summer, when temperatures are extreme. In this way, sampling was concentrated in periods of peak activity, when lizards could be found basking and always during the reproductive season (March to September) (Zamora-Camacho et al. 2013).

Lizards were sampled during four consecutive years, from 2010 to 2013. During sampling periods, two researchers went to the field one or two days a week, provided climatology was adequate and personnel were available. A total of 568 lizards were captured in the six plots altogether.

Individuals were captured by hand and transported to the laboratory facilities (at a distance of 50 to 110 kilometres depending on the sampling plot). Lizards were transported in cotton bags (in order to minimize stress by providing them a dark and well ventilated refuge) the same day. Once in the laboratory, lizards were kept in plastic terrariums (20×13×9cm centimetres) with pine tree bark as substrate. Water and food were provided *ad libitum* in the form of vitamins and mineral enriched aqueous gel and *Tenebrio molitor* larvae. A heating cable was placed in one of the sides of the terrarium to enable lizards to thermoregulate. The cable was turned on during the central hours of the day (from 12 to 4 pm) simulating heat peaks in the natural environment, with temperatures from 30 to 33 ºC (Carrascal & Díaz 1989). All terrariums were kept in a naturally illuminated room allowing the maintenance of natural circadian cycles of light and darkness. Lizards were released at the capture site within a week after capture without damage as a consequence of this study.

Body mass, sex and tail state (autotomized, regenerating or intact) of each lizard were recorded, as well as morphometric measurements such body length (SVL; snout-vent length) and hind limb length (HLL). Gravid state was also recorded for females. Body mass was measured with an electronic balance (Model Radwag, WTB200, accuracy 0.01 g), and SVL and HLL were measured with a metal ruler (accuracy 1 mm). Sex was characterized by femoral pores, more conspicuous in males (Iraeta et al. 2011). Gravid state was detected palpating the abdomen of females. Lizards were considered attacked by a predator when tail was regenerated or incomplete and non-attacked when tail was complete (see Medel et al. 1988). Autotomy of the tail is a defensive tactic used by numerous lizards to escape from predators when other strategies, such as fleeing or crypsis, have not been effective (Bellairs & Bryant 1985; Arnold 1994).

Sex, gravid state, SVL and body mass might influence the degree of exposure or vulnerability towards predators. Males are usually more active during the reproductive season (mating and guarding territory), which implies higher exposure times (Díaz, 1993). Sprint speed (thus also escape velocity) of gravid females is reduced in several reptile species (Shine 1980; Bauwens & Thoen 1981; Snell et al. 1988; Brodie III 1989a, b; Cooper et al. 1990; Sinervo et al. 1991), including our target species (Iraeta et al. 2010). Increased basking by gravid females may also result in an increased predation risk due to greater exposure time (Shine 1980; Andren 1985; Madsen 1987). Nevertheless, this could be compensated by gravid females being more cryptic (Bauwens and Thoen 1981; Andren 1985; Madsen 1987; Braña 1993). HLL can be considered as an indicator of the lizards' capability to escape from predators as longer hind limbs imply higher escape velocities (Bawens et al. 1995; Rubiño-Hispán 2012; Zamora-Camacho et al. 2012b).

To avoid the effect of possible ontogenetic shift in the characters measured, we considered only adult individuals in the analyses, condition established according to body size. We considered different minimum SVLs for each sex: that of the smallest male with orange commissure for males and that of the smallest gravid female for females. Both of these characters objectively reveal maturity. Thresholds were independently established for each sampling plot (Table 1).

Table1.- SVL (in mm) considered in classifying age categories by altitude.

Altitude	**Male SVL**	**Female SVL**
300	55	53
700	55	54
1200	55	56
1700	55	59
2200	62	65
2500	63	67

3.5.- Colour measurement

Colour measurements were performed in each individual with a spectrophotometer (Minolta CM-2600d/2500d). Colour measures consisted of the three chromatic values of the L*a*b* colour space of the *Commission Internationale d'Eclairage* (CIE). This colour space describes all the colours visible for most diurnal terrestrial vertebrates (Montgomerie 2006). The coordinate L* represents the Lightness of the colour, from black ($L^* = 0$) to white ($L^* = 100$); a* represents the position of the colour in the green-red gradient ($a^*<0$ when green and $a^*>0$ when red); b* represents the position of the colour in the blue-yellow gradient ($b^*<0$ when blue and $b^*>0$ when yellow). Measures were obtained from two points of the dorsum surface, one point at the pileus (2010 - 2013) and other at the middle of the back (2011 - 2013).

We also considered *Chroma* (colour saturation), $C^*=[(a^*)^2+(b^*)^2]^{1/2}$ (0 in the centre of the colour-space and increases according to the distance from the centre); and Hue angle, $H^*=(180^*\arctan2(b^*, a^*))/\pi$ (defined as starting at the +a* axis and is expressed in degrees, 0º for +b* [yellow], 90º for +a* [red], ±180º for –b* [blue], and -90º for –a*[green]). As a result an L*C*H* could be also obtained. The L*C*H* colour space has the advantage of expressing pure colouration in a single value (H*) allowing a more intuitive and comprehensive analysis. All variable transformations were carried out following CIE normative.

Environment elements' colouration sampling took place between May and July of 2013, two or three times a week depending on climatology and personnel availability. During the sampling sessions we took photographs of the surroundings wherever a lizard was observed basking. Photographs were taken with a Canon 1000D reflex camera with an 18 - 55 millimetre objective (nevertheless all photographs were taken with the objective set to 24 millimetres). Aperture was set to F22 to ensure the image would be focused within a wide range of depths providing a clear image despite the irregularities of the surroundings. White balance was calibrated by means of photographing a white paper in the sun and setting its colour as pure white before each sampling session. A tripod was used to ensure distance was constant (60 centimetre) and orientation orthogonal to the ground. Only aperture time was adjusted for each photograph so that all colours would be represented. Additionally, a standard colour chart was placed in the terrain so that it would appear in each photograph to allow posterior standardization of colour measurements. Also, an UV-filter was used for noise reduction, as lizards' dorsal colouration never includes prominent reflection for these wavelengths (Pérez i

Lanuza 2012), and RGB images do not allow their analysis. A total of 167 photographs were taken across the six sampling plots.

We used Adobe Photoshop CS5 software to process colouration in all the photographs. During image processing and previous to measurement, white balance was newly calibrated and, thus, standardized in all photographs by sampling the grey scale of the colour chart present in each image with the white balance calibration function (curve layer). Then, in each photograph, each category of elements of the environment was sampled in twenty haphazardly established points, employing the Eyedropper Point Sample tool. Four distinct categories of elements of the environment were established for measurement: ground, rocks, fresh vegetation, and dry vegetation. Point width was set to 5 x 5 pixels, each measurement corresponding to the mean colouration of 25 pixels. Despite images where originally in RGB colour space, we processed the photographs directly in L*a*b* CIE colour space to be comparable with lizard colouration measurements made with the spectrophotometer. Difference between lizards' and environment elements' colouration was calculated with the formula $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ proposed by the CIE.

3.6.- Data analysis

We conducted a set of tests in an attempt to answer all the questions proposed to determine the relationship between the environmental elements' and lizards' dorsal colouration along the altitudinal gradient (see section 2). All analyses were performed by using software R 2.15.2 (R Development Core Team 2012).

3.6.1.- How does environmental elements' colouration vary with altitude?

We tested the relationship among different colour components (Lightness "L*", Chroma "C*" and Hue angle "H*") and different environment elements' colouration (ground, rocks, fresh vegetation, and dry vegetation), with Pearson's correlation. Then, in order to test a possible variation of the environment's colouration along altitudinal gradient, we ran analyses of the variance (ANOVA) with colour components as dependent variables and altitude (each of the six sampling plots) as factor.

3.6.2.- How does lizards' dorsal colouration vary with altitude?

We tested the relationship among colour components of dorsal lizards' surface with Pearson's correlation. In order to eliminate repetitive information (highly correlated variables), pileus data were disregarded as they were highly correlated with back colouration. The latter was selected over the first because it comprises a larger area of the animals' visible surface. Then we ran ANOVA test to check for possible variance of the lizards' dorsal colouration along the altitudinal gradient using colour traits as dependent variables and altitude as factor.

3.6.3.- Is there any relation between the variation in environmental colouration and lizards' dorsal colouration?

For lizard and environmental data to be comparable, the mean of each variable was determined for each altitude. Then, a Spearman's correlation, as recommended by Siegel & Castellan (1988) for small datasets, was run in order to examine the relation between environmental elements' colouration and that of the lizards' dorsum. We used each one of the six plots as statistical units in the analysis.

3.6.4.- How does the difference between lizards' dorsal colouration and the colour of their environment vary in altitude?

Difference between each of the lizards' dorsal colouration and the mean colouration of each environmental element for each altitude was obtained. This difference (ΔE) was calculated following the formula $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ by the CIE. We tested the relationship among colour difference variables, and of these with lizard's back CIE colour space components using Pearson's correlation. Then, we tested if ΔE* depended or not on altitude with an ANOVA test using colour difference as dependent variable and altitude as factor.

3.6.5. - How are lizards' colouration and its difference with environmental elements' colouration related to lizards' biological features?

We tested the relationship among quantitative biological feature variables (SVL, mass and HLL), and of these with colour difference variables, using Pearson's correlation. We also tested qualitative variables by means of a Spearman's correlation, using each one of the six plots as statistical units. Because sex, gravid state and tail status are categorical variables, we considered 1 and 0 values for each category in the next way: 1 was assigned to "male", "gravid" and "predated" (here we mean unsuccessfully attacked by a predator); 0 was assigned to "female", "non-gravid" and "non-predated" states. A value of 1 was attributed to those states which a priori imply higher predation risk or susceptibility. Hence, mean values of each plot corresponded with the frequency of males, gravid females and tail loss, allowing these variables to be included in correlation analysis as quantitative values.

Finally, in order to explore the relationship among colour differences (ΔE*) and lizards' biological features, we ran multi-way ANCOVA tests introducing colour difference for each individual as dependent variable and altitude, reproductive status, body size (principal component obtained from the combination of SVL and mass; see section 4.5.1), HLL and tail state as factor and covariates. Reproductive status was the result of combining the two level factors sex and gravid state into one with three levels: male, non-gravid female, and gravid female. Tail status factor in this analysis also presented three levels (complete, regenerating and autotomized) in order to avoid information loss.

4.- RESULTS

4.1.- How does environmental elements' colouration vary with altitude?

Correlations among environmental colour variables were relatively low (Table 2). Thus, all variables were considered in posterior analyses, as they were not redundant. Highly significant correlations were found between dry and fresh vegetation's Lightness, dry vegetation's and both rock's and ground's Chroma, and ground's and rock's Hue angle. Secondarily, correlation between dry vegetation's and ground's Lightness, dry and fresh vegetation's Chroma, ground's and rock's Chroma, and ground's and both fresh and dry vegetation's Hue angle were also significant.

ANOVA tests indicated only the three components of the colour space of ground colouration significantly varied with altitude (Figure 6): Lightness ($F_{(5,90)}$= 7.45, $P<0.001$); Chroma ($F_{(5.90)}$= 2.73, $P<0.05$); and Hue angle ($F_{(5,90)}$= 6.68, $P<0.001$), the remaining substrate colour components showing no significant variation with altitude. Residuals from ANOVA tests were normally distributed and homoscedastic.

Table 2.- Pearson's correlation coefficients (*r*) for environmental element variables against each other for each component of the L*C*H* CIE colour space. Test significance is indicated as follows: *= *P*<0.05; **= *P*<0.01; ***= *P*<0.001.

	Ground	Rock	Fresh vegetation	Dry vegetation
Lightness				
Ground	1.00			
Rock	0.30	1.00		
Fresh vegetation	-0.01	0.19	1.00	
Dry vegetation	0.23 *	0.19	0.36 ***	1.00
Chroma				
Ground	1.00			
Rock	0.40 *	1.00		
Fresh vegetation	0.13	-0.17	1.00	
Dry vegetation	0.46 ***	0.50 ***	0.26 *	1.00
Hue angle				
Ground	1.00			
Rock	0.50 ***	1.00	-0.17	
Fresh vegetation	0.24 *	-0.17	1.00	
Dry vegetation	0.29 *	0.07	0.06	1.00

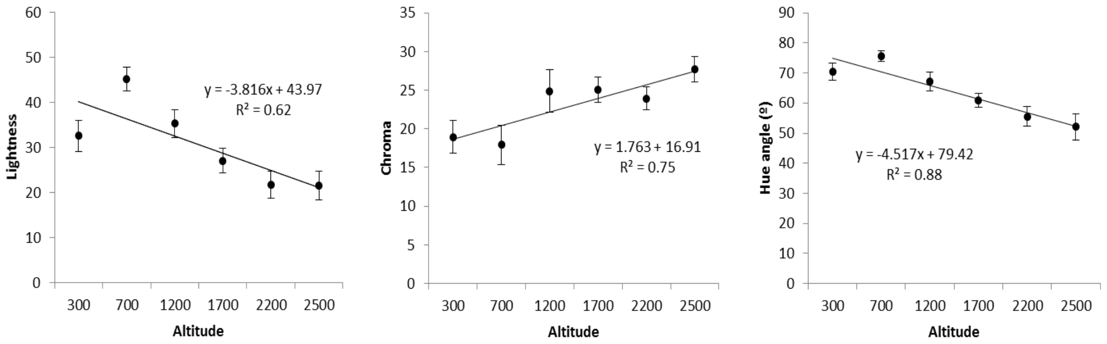

Figure 6.- Mean (points), standard error bars, and regression fit of each L*C*H* CIE colour space component of the ground's colouration across altitudes. Lightness ranges from black (L* = 0) to white (L* = 100); Chroma represents colour saturation; Hue angle indicates yellow at 0º, red at 90º, blue at ±180º, and green at -90º.

4.2.- How does lizards' dorsal colouration vary with altitude?

Correlations between lizards' back and pileus colouration variables were highly significant for the three colour components of the L*C*H* CIE colour space: Lightness (r=0.69, n=369, P<0.001); Chroma (r=0.66, n=369 P<0.001); Hue angle (r=0.68, n=369, P<0.001). Because of this, only back's colouration was included in posterior analyses. Lizards' back colouration was chosen over pileus' as it occupies a significantly larger area of the dorsal surface of the animal and, thus, is more representative.

The three components of lizard back colour (L*C*H*) significantly varied with altitude (Figure 7): Lightness ($F_{(5,366)}$= 12.92, P<0.001); Chroma ($F_{(5,366)}$= 17.26, P<0.001); and Hue angle ($F_{(5,366)}$= 7.60, P<0.001).

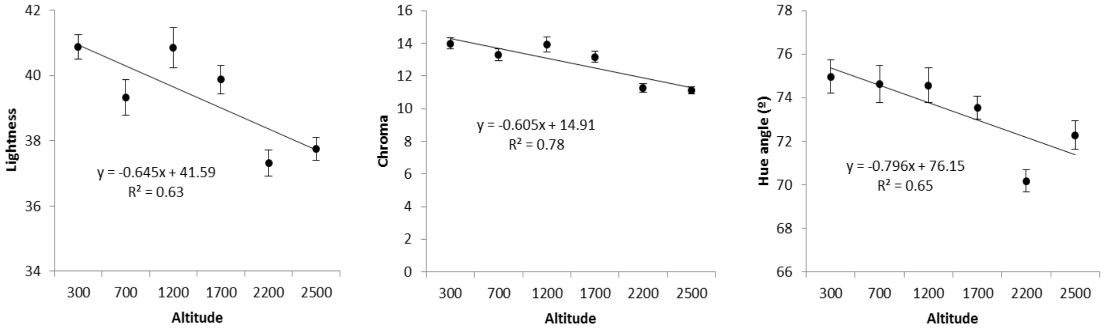

Figure 7.- Mean, standard error bars, and regression fit of each L*C*H* CIE colour space component of lizards' back colouration across altitudes. Lightness ranges from black (L* = 0) to white (L* = 100); Chroma represents colour saturation; Hue angle indicates yellow at 0º, red at 90º, blue at ±180º, and green at -90º.

4.3.- Is there any relation between the variation in environmental colouration and lizards' dorsal colouration?

Lizards' back Lightness tended to be correlated with both ground's (r_S=0.54, *n*=6, *P*=0.27) and rock's (r_S=0.60, *n*=6, *P*=0.21) Lightness, but not significantly (Figure 8). Similar but negative tendencies were observed for ground's (r_S=-0.60, *n*=6, *P*=0.21) and fresh vegetation's (r_S=-0.66, *n*=6, *P*=0.16) Chroma. Lastly, lizards' back Hue was highly and significantly correlated with ground's (r_S=0.89, *n*=6, *P*<0.05) and fresh vegetation's (r_S=0.83, *n*=6, *P*<0.05) Hue (Figure 8). No other correlations between the lizards' back colouration and the colouration of the elements of the environment were found. Therefore, lizard Hue correlated with their populations' ground and fresh vegetation Hue.

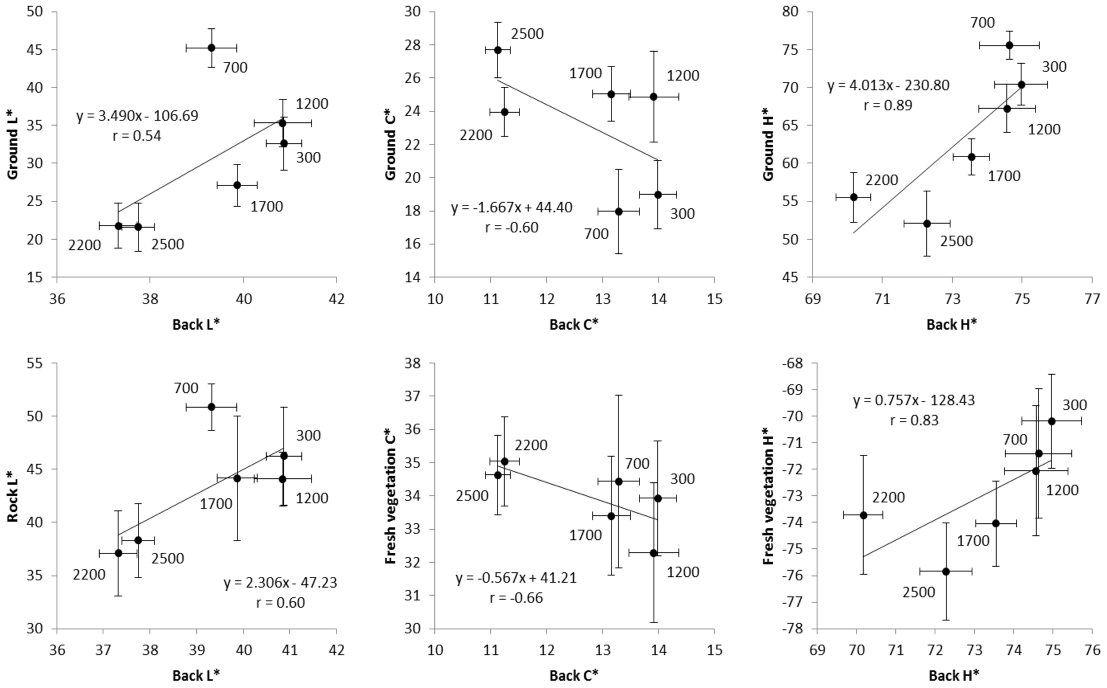

Figure 8.- Regressions for Lizards' back colouration against ground's and rock's colouration for the three components of the L*C*H* CIE colour space. Lightness, Chroma and Hue angle correspond to the L*C*H* CIE colour space. Lightness ranges from black (L* = 0) to white (L* = 100); Chroma represents colour saturation; Hue angle indicates yellow at 0º, red at 90º, blue at ±180º, and green at -90º. Mean value and standard error bars for each plot (300, 1200, 1700, 2200 and 2500 m. asl) are represented. Standard error bars are represented in vertical for environmental elements' chromatic values, and in horizontal for lizards' back.

4.4.- How does the difference between lizards' dorsal colouration and the colour of their environment vary in altitude?

Highly significant correlations were found between all colour difference variables except for back vs. ground (Table 3). The latter only showed a significant but low correlation against the back versus fresh vegetation colour difference variable.

Table 3.- Pearson's correlation coefficients (*r*) for colour difference variables against each other. Test significance is indicated as follows: *= *P*<0.05; **= *P*<0.01; ***= *P*<0.001.

	Back vs ground	Back vs rock	Back vs fresh veg.	Back vs dry veg.
Back vs ground	1.00			
Back vs rock	0.08	1.00		
Back vs fresh veg.	0.17 ***	0.29 ***	1.00	
Back vs dry veg.	0.03	0.68 ***	0.71 ***	1.00

ANOVA tests indicated that population was a significant factor explaining variance in colour difference along the gradient: ground ($F_{(5,366)}$= 342.10, *P*<0.001), rocks ($F_{(5,366)}$= 75.66, *P*<0.001), fresh vegetation ($F_{(5,366)}$= 67.79, *P*<0.001) and dry vegetation ($F_{(5,366)}$= 31.31, *P*<0.001). Although there were differences among populations (Figure 9), a clear increase with altitude was only observed for the back vs. ground colour difference variables.

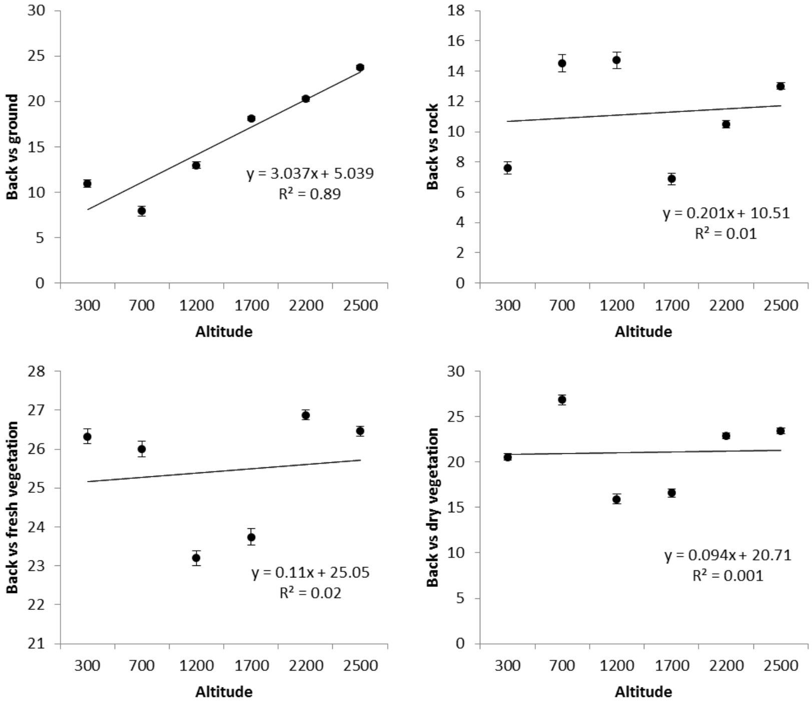

Figure 9.- Mean, standard error bars, and regression fit across altitudes for the colour differences between lizards' back colouration and environmental elements' colouration.

We also examined how the variance in the difference in colour varied with elevation. We found that variance for all colour difference variables followed a clear pattern along the gradient, decreasing with altitude (Figure 10), however the correlation with altitude was only significant, again, for the difference between dorsal colour and both ground rock colouration (Table 4).

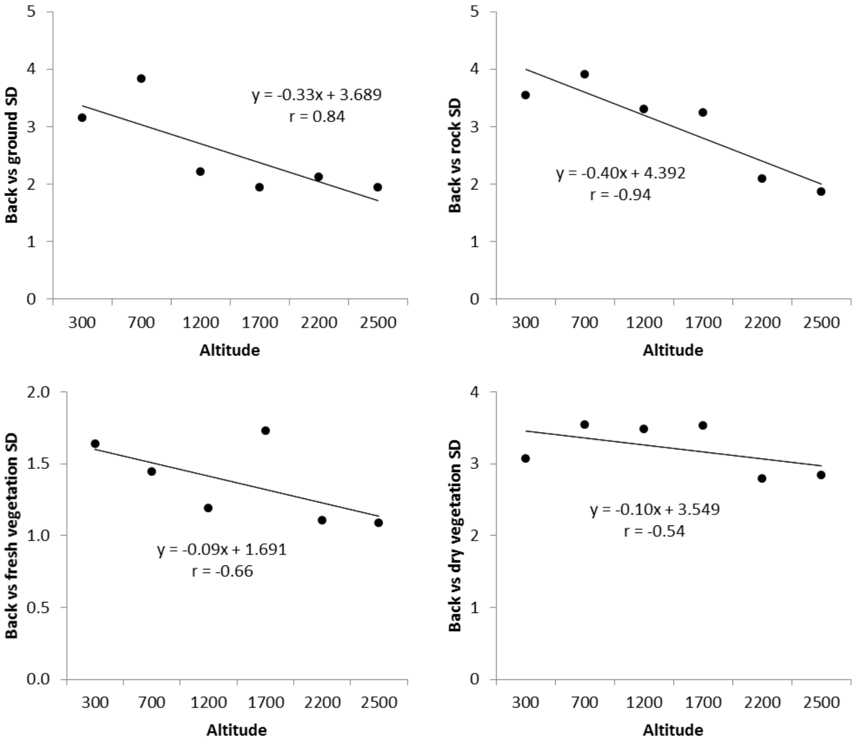

Figure 10.- Standard deviation and regression line across altitudes for all colour difference variables.

Table 4.- Spearman's correlation coefficients (r_s) for colour difference variables' standard deviation against each other and altitude. Test significance is indicated as follows: *= $P<0.05$; **= $P<0.01$; ***= $P<0.001$.

	Altitude	Back vs ground SD	Back vs rock SD	Back vs fresh vegetation SD	Back vs dry vegetation SD
Altitude	1.00				
Back vs ground SD	-0.84 *	1.00			
Back vs rock SD	-0.94 ***	0.90 **	1.00		
Back vs fresh vegetation SD	-0.66	0.23	0.60	1.00	
Back vs dry vegetation SD	-0.54	0.41	0.71	0.66	1.00

4.5.- How are lizards' colouration and its difference with environmental elements' colouration related to lizards' biological features?

4.5.1.- Lizard's colouration vs. biological features

All quantitative biological features were significantly correlated: SVL *vs*. HLL (r=0.47, n=491, P<0.001), SVL *vs*. mass (r=0.90, n=486, P<0.001), HLL *vs*. mass (r=0.50, n=484, P<0.001). This was to be expected as the characters evaluated possess a high influence over each other. Large lizards will typically have a proportionated body length, mass, and hind limb length (allometric relationships). Mass was significantly correlated with both SVL and HLL, and presented temporal variability (i.e. gravid state, nutritional, fat accumulation). Thus, in order to avoid multi-colinearity, a principal component analysis factor, called "size", was obtained from the combination of body mass and SVL. Correlation with HLL did not vary as a result (Table 5).

Table 5.- Pearson's correlation coefficients (r) for biological feature variables against each other and each L*C*H* CIE colour space component of lizards' back colouration. Test significance is indicated as follows: *= P<0.05; **= P<0.01; ***= P<0.001.

		Size	HLL
Size		1.00	
HLL		0.50 ***	1.00
Lizards' back	**L***	-0.16 ***	-0.11 *
	C*	-0.27 ***	-0.03
	H*	-0.18 ***	-0.06

Size showed a significant negative correlation with all three lizard's back colour components (Table 5; Figure 11): Lightness, Chroma and Hue angle. Lizard's back Lightness was also significantly and negatively correlated with HLL (Figure 11). ANOVA tests revealed a significant effect of altitude on the two variables, both fitting polynomial regressions (Figure 12): Size ($F_{(5,480)}$= 120.84, P<0.001) and HLL ($F_{(5,480)}$= 16.79, P<0.001).

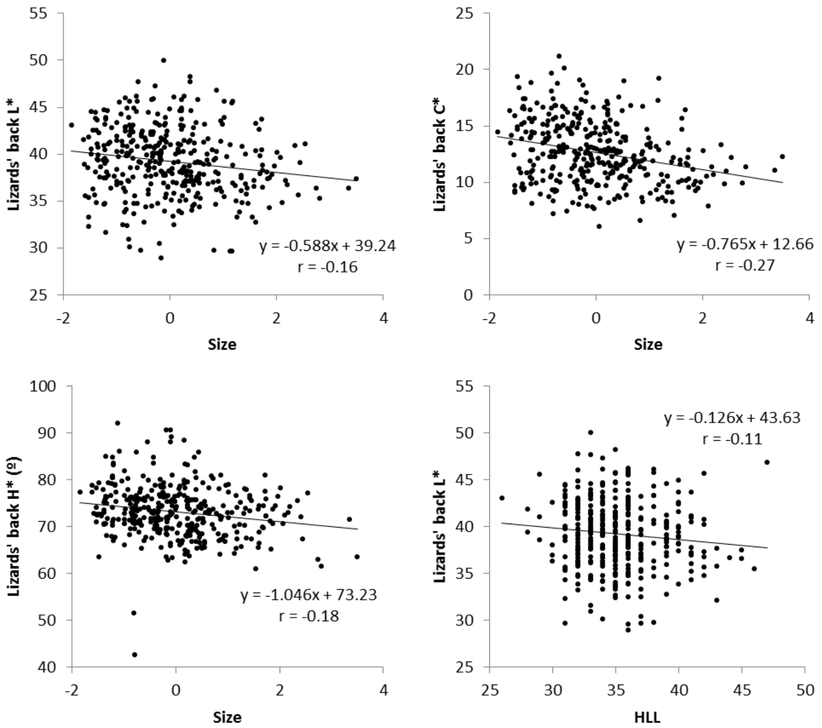

Figure 11.- Regressions for lizards' back colouration against size biological feature variable for the three components of the L*C*H* CIE colour space, and of lizards' back Lightness and HLL. Lightness, Chroma and Hue angle correspond to the L*C*H* CIE colour space. Lightness ranges from black (L* = 0) to white (L* = 100); Chroma represents colour saturation; Hue angle indicates yellow at 0º, red at 90º, blue at ±180º, and green at -90º.

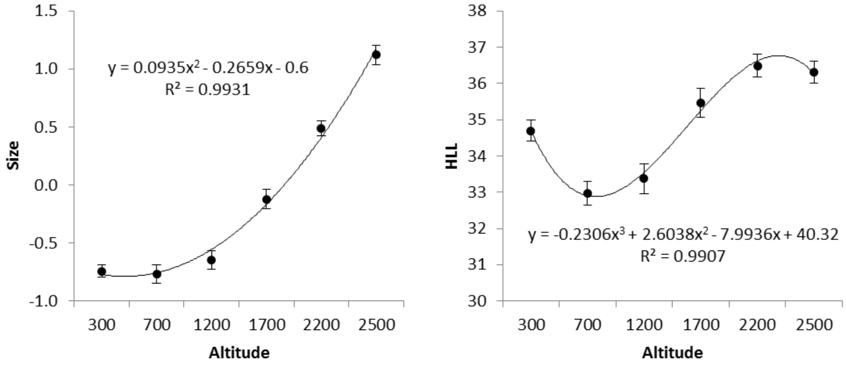

Figure 12.- Mean, standard error bars, and regression fit (polynomials) across altitudes for size and HLL biological feature variables.

In order to ascertain the relative effect of altitude and body size on dorsal colouration, we performed multiple regression models with the two variables size and altitude, as well as HLL, introduced as predictors of colouration. The models systematically revealed significant influence of altitude on lizards' colouration (Table 6).

Table 6.- Results for the multiple regression models performed with each L*C*H* CIE colour space component of lizards' back colouration as dependent variable and altitude, size and HLL as predictors (* : $P<0.05$; ** : $P<0.01$; *** : $P<0.001$).

Variable	β estimate	Std. Error	t-Value	Pr(>|t|)
Lightness				
Intercept	0.023	0.049	0	0.647
Altitude	-0.448	0.070	-6394	<0.001 ***
Size	0.176	0.076	2318	0.021 *
HLL	-0.072	0.058	-1227	0.221
Chroma				
Intercept	0.005	0.048	0	0.920
Altitude	-0.420	0.068	-6148	<0.001 ***
Size	-0.037	0.074	-1	0.617
HLL	0.097	0.057	1705	0.089
Hue angle				
Intercept	0.016	0.051	0	0.756
Altitude	-0.248	0.072	-3428	<0.001 ***
Size	-0.039	0.078	0	0.619
HLL	0.023	0.060	0	0.704

Gravid female frequency (over total females) was significantly and highly correlated with lizards' back Lightness and Hue angle (Table 7). Correlation with Hue angle was not significant but also high. Male and tail loss frequencies were also significantly and highly correlated against each other.

Table 7.- Spearman's correlation coefficients (*r*) for biological feature variables against each other and against each L*C*H* CIE colour space component of lizards' back colouration. Test significance is indicated as follows: *= $P<0.05$; **= $P<0.01$; ***= $P<0.001$.

	Male frequency	Gravid female freq.	Tail loss frequency
Male frequency	1.00		
Gravid female freq.	-0.09	1.00	
Tail loss frequency	0.91 *	0.26	1.00
Lightness	0.09	-0.89 *	-0.26
Chroma	-0.21	-0.83 *	-0.43
Hue angle	-0.06	-0.71	-0.26

An ANCOVA indicated that lizards' back colour Lightness and Chroma were significantly affected by reproductive condition (Table 8; Figure 13). Also, tail status had a significant effect over lizards' back Hue angle (Figure 13). As previously described when the effect of altitude on lizard's dorsal colouration was examined (see section 4.2; see also Reguera et al. 2014), altitude was a significant factor in all cases ($P<0.001$).

Variance across altitude for all categories of these two biological feature variables fitted for a polynomial regression (Figure 14).

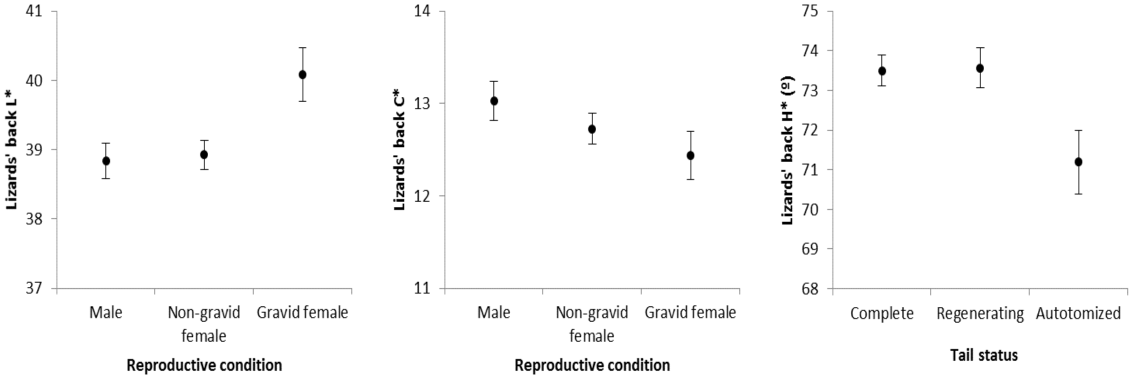

Figure 13.- Mean and standard error bars of lizards' back Lightness and Chroma for each category of the sexual condition biological feature variable, and for lizards' back Hue angle for each category of the tail state biological feature variable. Lightness, Chroma and Hue angle correspond to the L*C*H* CIE colour space. Lightness ranges from black (L* = 0) to white (L* = 100); Chroma represents colour saturation; Hue angle indicates yellow at 0º, red at 90º, blue at ±180º, and green at -90º.

Table 8.- Results for the ANCOVA test performed with altitude as factor, with SVL, HLL, tail status and sex or gravid status as cofactors; and lizards' back colouration as dependent variable (* : *P*<0.05; ** : *P*<0.01; *** : *P*<0.001).

Variable	*n*	*df*	*F*	*P*-value
			Lightness	
Altitude	361	5	13.81	<0.001 ***
Size	361	1	1.82	0.178
HLL	361	1	0.07	0.794
Reproductive condition	361	2	6.82	0.001 **
Tail status	361	2	0.35	0.702
			Chroma	
Altitude	361	5	18.21	<0.001 ***
Size	361	1	0.12	0.727
HLL	361	1	0.02	0.891
Reproductive condition	361	2	7.41	<0.001 ***
Tail status	361	2	0.31	0.731
			Hue angle	
Altitude	361	5	7.99	<0.001 ***
Size	361	1	0.15	0.703
HLL	361	1	0.12	0.734
Reproductive condition	361	2	0.86	0.424
Tail status	361	2	6.86	0.001 **

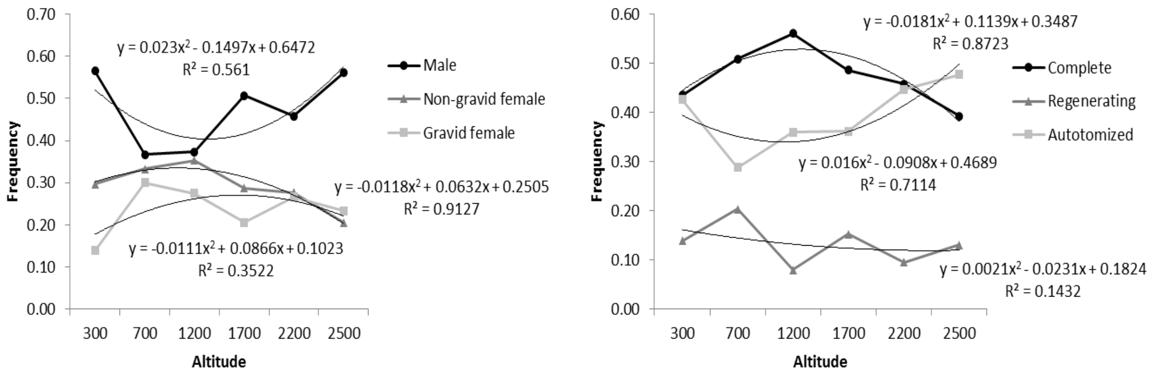

Figure 14.- Frequency and regression fit (polynomial) of each reproductive condition and tail status (biological features) category.

4.5.2.- Colour differences vs. lizards' biological features

A highly significant correlation was found between the back versus ground colour difference variable and both size and HLL, and between back versus fresh vegetation colour variable and size (Table 9; Figure 15). Secondarily, HLL was significantly correlated with the back versus fresh vegetation colour difference variable (Table 9; Figure 15). In fact, HLL remained significantly and the difference in colouration between lizards dorsum and rock ground, even after controlling for altitude and size (Table 10).

Table 9.- Pearson's correlation coefficients (*r*) for biological feature variables against each colour difference variable. Test significance is indicated as follows: *= *P*<0.05; **= *P*<0.01; ***= *P*<0.001.

	Size	**HLL**
Back vs ground	0.63 ***	0.26 ***
Back vs rock	0.06	-0.13 *
Back vs fresh veg.	0.19 ***	0.00
Back vs dry veg.	0.10	-0.05

Table 10.- Results for the multiple regression performed with each colour difference variables as dependent variable and altitude, size and HLL as predictors (* : $P<0.05$; ** : $P<0.01$; *** : $P<0.001$).

Variable	*β* estimate	Std. Error	*t* -Value	Pr(>|*t* |)
			Back vs ground	
Intercept	0.001	0.026	0	0.963
Altitude	0.842	0.038	22387	<0.001 ***
Size	0.061	0.041	1493	0.136
HLL	0.009	0.031	0	0.763
			Back vs rock	
Intercept	-0.011	0.051	0	0.825
Altitude	0.293	0.072	4050	<0.001 ***
Size	-0.063	0.078	-1	0.421
HLL	-0.172	0.060	-2853	0.005 **
			Back vs fresh vegetation	
Intercept	0.004	0.051	0	0.942
Altitude	-0.093	0.073	-1274	0.204
Size	0.324	0.079	4120	<0.001 ***
HLL	-0.133	0.061	-2201	0.028 *
			Back vs dry vegetation	
Intercept	-0.003	0.052	0	0.952
Altitude	0.035	0.075	0	0.641
Size	0.136	0.081	1683	0.093
HLL	-0.121	0.062	-1950	0.052

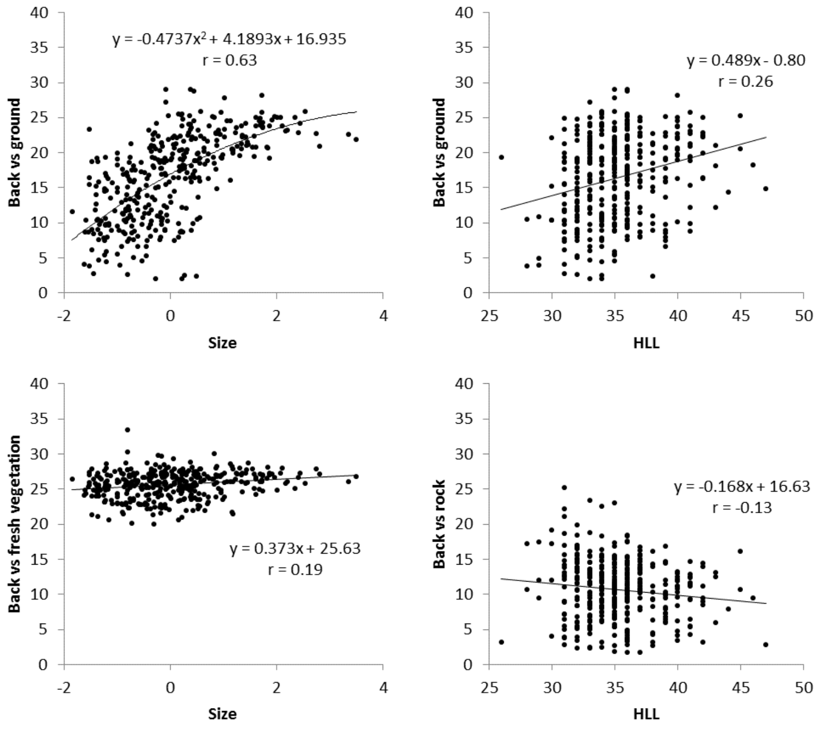

Figure 15.- Scatterplot and regression fit of Lizards' back vs. ground colouration difference variable against lizards' size and HLL (biological features), back vs. fresh vegetation variable against size, and back vs. rock variable against HLL.

Despite correlation of colour difference variables against male, gravid female, and tail loss frequency of each altitude were not significant, correlation coefficients were high (Table 11). The backs of individuals with predated tail status (regenerating or autotomized) and gravid females showed greater differences with environmental colouration (Figures 16, 17). ANCOVA tests revealed a significant effect of reproductive condition on the three colour difference variables (Table 12; Figure 17). Significance for the back vs. ground colour difference was much higher than for the remaining.

Table 11.- Spearman's correlation coefficients (*r*) for biological feature variables against each colour difference variable. Test significance is indicated as follows: *= *P*<0.05; **= *P*<0.01; ***= *P*<0.001.

	Male frequency	Gravid female freq.
Back vs ground	0.44	0.60
Back vs rock	-0.65	0.43
Back vs fresh veg.	0.47	0.54
Back vs dry veg.	0.06	0.60

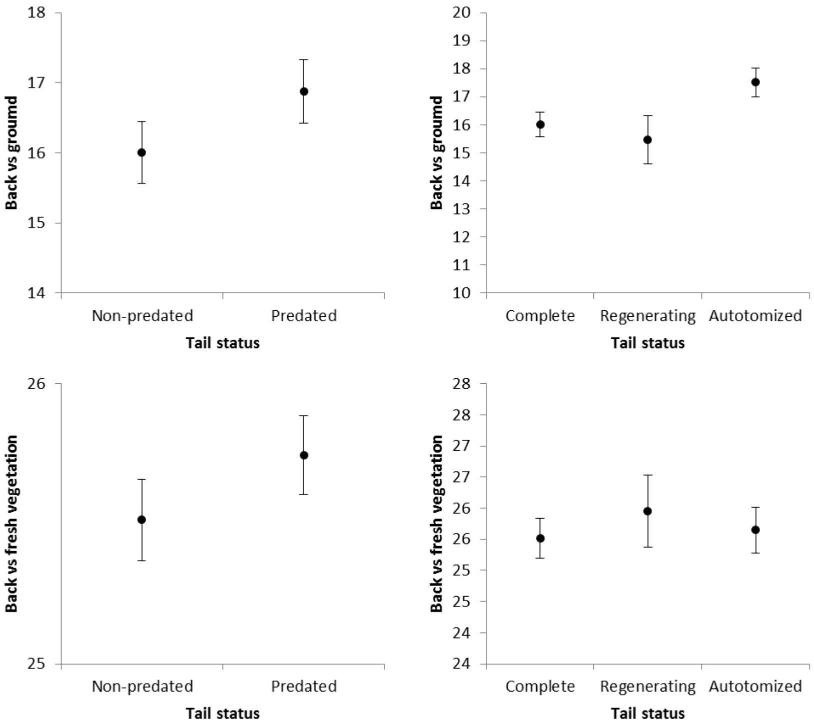

Figure 16.- Mean and standard error bars of each colour difference variable for each category of the tail status biological feature variable.

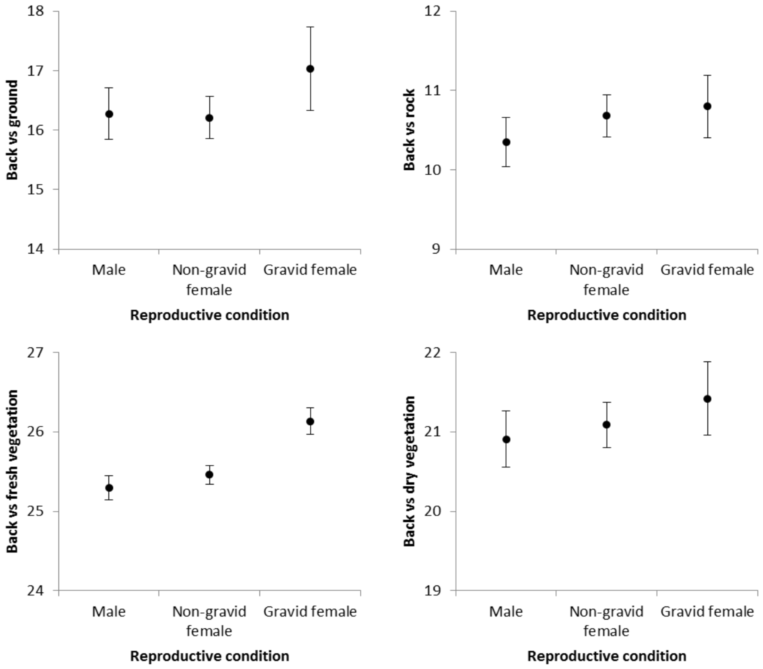

Figure 17.- Mean and standard error bars of each colour difference variable for each category of the sexual condition biological feature variable.

Table 12.- Results for the ANCOVA test performed with altitude as factor; SVL, HLL, reproductive condition and tail status as cofactors; and the colour difference between the lizards' back colouration and the different elements of their environment (colour difference variable) as dependent variable (* : *P*<0.05; ** : *P*<0.01; *** : *P*<0.001).

Variable	*n*	*df*	*F*	*P*-value
		Back vs ground		
Altitude	361	5	339.69	<0.001 ***
Size	361	1	0.02	0.883
HLL	361	1	0.17	0.681
Reproductive condition	361	2	3.93	0.020 *
Tail status	361	2	0.76	0.467
		Back vs rock		
Altitude	361	5	86.47	<0.001 ***
Size	361	1	2.84	0.093
HLL	361	1	0.01	0.921
Reproductive condition	361	2	5.69	0.004 **
Tail status	361	2	0.06	0.941
		Back vs fresh vegetation		
Altitude	361	5	70.80	<0.001 ***
Size	361	1	0.07	0.785
HLL	361	1	0.04	0.848
Reproductive condition	361	2	15.89	<0.001 ***
Tail status	361	2	1.07	0.343
		Back vs fresh vegetation		
Altitude	361	5	91.24	<0.001 ***
Size	361	1	0.64	0.424
HLL	361	1	0.00	0.995
Reproductive condition	361	2	4.33	0.014 *
Tail status	361	2	0.09	0.912

5.- DISCUSION

5.1.- How does environmental elements' colouration vary with altitude?

Although there were some significant correlations among environmental variables, correlation coefficients were low (r<0.5). This suggests that the colouration of each element in the environment varied independently. Only ground colouration significantly varied with altitude, with increasing Chroma and decreasing Hue and Lightness, corroborating the existence of the perceived ground colour gradient. Decreasing Lightness variation indicated that ground colouration gets darker as altitude increases. Therefore, this finding could explain why lizards darken with altitude (see Reguera et al. 2014), as a consequence of crypsis. This point will be discussed below. Chroma and Hue angle tendencies indicated ground colouration shifts from red to yellow and gets more saturated with altitude.

5.2.- How does lizards' dorsal colouration vary with altitude?

Results revealed that lizards' dorsal colouration is uniform, existing no significant difference between pileus and back. All three Chromatic values of lizards' dorsal colouration varied significantly in altitude, confirming the existence of an altitudinal gradient already described (Reguera et al. 2014). Lizards darkened with altitude, which is consistent with the thermoregulation as well as the UV protection hypotheses (see Reguera et al. 2014). However, given that ground colouration also darkened with altitude, changes in lizard dorsal Lightness are also consistent with the crypsis hypothesis. Moreover, Hue angle followed the same tendency in altitude as ground's respective CIE colour space component and within the same range of values. Thus, lizards' back colour not only gets darker with altitude but also appears to change parallel to ground colouration. This colour matching would imply a cryptic strategy being in play, and points out predation as the main selective pressure behind the phenotypic gradient.

Opposite tendencies between ground's and lizards' back colouration were observed for the Chroma chromatic value. Nevertheless, variation in colour saturation may not significantly influence crypsis, especially within the range of variation detected during our study. Colour saturation indicates the intensity of colour. A small alteration in Chroma hence does not necessarily imply a perceptible change in colour, which is mainly defined by tone (Hue angle) and brightness (Lightness). At low altitude, lizards' and grounds' Chroma values were very proximate probably rendering the difference undetectable by predators. Chroma values increasingly diverge as altitude increases (following the same tendency as colour difference variables discussed below).

5.3.- Is there any relation between the variation in environmental colouration and lizards' dorsal colouration?

Correlations found between lizards' back and environmental elements' colouration confirm and reinforce the assumptions made in the previous section. High correlation was found between lizards' back and ground's both Lightness and Hue angle values, strongly suggesting crypsis, regardless only the latter was significant. The strong correlation between Hue angle in lizards and ground is not predicted by the thermoregulation or UV-radiation damage protection hypothesis. According to these hypotheses, Hue angle would not have to vary in the same way as the grounds', as both mechanisms only imply a higher concentration of melanins (eumelanin) in lizards' skin (darker individuals; lower Lightness values) with increasing altitude. Exact colour matching, which in this case involves other pigments (such as pheomelanins or carotenes) is only necessary for crypsis. In order to elucidate the role of each pigment in lizards' colouration, further research is needed, especially at the molecular level.

A significant and high correlation was also found between fresh vegetation's and lizards' dorsal Hue angle. *P. algirus*, as explained before, usually selects the ground surrounding shrubs and bushes as basking sites (Martín & López 1995). Thus vegetation colour may also have an influence over cryptic colouration, especially considering that avian visual predators have a zenithal point of view. Nevertheless, vegetation Hue angle values correspond to green colouration, while lizards' Hue angle varied within the yellow-red range. Therefore, the reason for this correlation remains unknown.

Additionally, a high (but not significant) correlation between lizards' back and rock's Lightness is also reported. This suggests colour matching could be partially oriented towards rocks. Despite not being its preferred basking sites, it is not rare to find *P. algirus* basking over them (personal observation). Rocky surfaces are often more exposed to predators but have greater thermal efficiency than the ground. Rocks heat up faster in the morning and preserve heat longer during the late hours of the day. Nevertheless, care has to be taken in this interpretation, as the correlation between rocks' and lizards' back Hue angle was non-significant.

5.4.- How does the difference between lizards' dorsal colouration and the colour of their environment vary in altitude?

Our results revealed variation between populations for all colour difference variables (lizards' dorsum against each environmental element). Colour difference for rock, fresh vegetation and dry vegetation were significantly correlated with each other and varied in a similar way along the gradient, although they did not follow a clear pattern. In contrast, lizards' back *versus* ground colour difference did follow a clear pattern along the gradient, increasing with altitude. This not only supports that colour matching in this species is oriented mainly towards ground colouration, but also suggests decreasing predation pressure with increasing altitude. This was expected as a lower abundance of predators was observed in the upper sampling stations during field work and other studies describe the same pattern of decaying predation intensity with altitude (Van Damme et al. 1989; Fox et al. 1994).

The smallest colour differences in our study were found for ground at low altitude sampling stations and for rocks in some of them. This further supports the cryptic hypothesis, as colour matching appears once again oriented towards *P. algirus'* preferred basking sites (ground and secondarily rocks).

Nevertheless, and contrary to what was expected, variance for all colour difference variables followed a clear pattern along the gradient, decreasing with altitude. If predation pressure at low altitudes is more intense, variance in colouration difference should be more constrained. However, this tendency could be explained if we also took into account thermoregulation and/or UV-protection effects on colouration. Both of these mechanisms gain relevance with altitude, as temperature decreases and UV radiation increases. Considering that predation pressure seems to decrease with altitude, it is likely there is a shift in the relative importance of other selective pressures along the gradient. This shift would imply thermoregulation and/or UV-protection having a more restrictive effect on lizards' dorsal colouration than predation pressure. Thermoregulation and UV-protection select for enough concentration of melanin to either efficiently thermoregulate or effectively protect against UV-radiation damage without incurring in excessive metabolic costs, which are significant for this pigment. Thus, optimum melanin concentration is expected to vary within a narrower range of values.

On the other hand, optimum cryptic colouration can admit a higher degree of variation as ground colouration also varies within the same population (see below about the existence of different strategies of crypsis). Hence, colour matching could be directed to a wider range of colourations, which does not necessarily imply significant differences in terms of metabolic costs (as they are probably the result of complex combinations of different pigments) or survival. Lizards' dorsum would be restricted to relatively inconspicuous melanin-rich colourations, so thermoregulatory and UV-protection requirements would be met; while at low altitudes melanin concentration would present greater variation due to a decrease in importance of these needs, allowing a greater diversity of colourations.

5.5.- How are lizards' colouration and its difference with environmental elements' colouration related to lizards' biological features?

Results described a significant correlation between size and all three Chromatic values of lizards' dorsal colouration. Individuals display darker, less saturated and more yellow colourations as size increases, in the same way they do with altitude. This tendency was expected, as body size increases with altitude. Colour differences were also correlated with size.

In addition, HLL was greater for dark individuals. This could be caused by HLL's allometric relationship with body size, but could also be interpreted as a shift from a cryptic to a fleeing anti-predator strategy with altitude. The former hypothesis was discarded by multiple regression analyses, which give support to the latter hypothesis. When controlling for altitude and body size, HLL was significantly and negatively correlated with rock colour difference variables (HLLs increased with decreasing colour matching). Nevertheless, no differences were observed in sprint speed along the gradient (Zamora-Camacho et al. 2012b), although lizards' in the uppermost stations had relatively shorter hind limbs (in relation to body size) than in the rest. Given that HLL is directly related to escape speed in this lizard (Bauwens et al. 1995), this finding suggests the possibility of different anti-predatory strategies in our population. Faster lizards have more probability of survival to a predator attack, and thus selection on crypsis is relaxed in these specimens.

Altogether, these findings suggest two possibilities. On the one hand, the larger size of lizards at high altitudes (as a result of increased resource abundance) could be increasing their detectability by predators. As a consequence, the cryptic strategy would decrease in importance due to ineffectiveness, favouring the effect of other selective pressures over lizards' dorsal colouration, such as thermoregulation or UV-protection, as suggested previously. This would generate the increase in colour difference variables observed. On the other hand, the cryptic strategy could be decreasing in importance with altitude due to a decrease in predator abundance. In this case, the increase in size with altitude could be a consequence of low predation rates, as lizards would be able to increase exposure time optimizing their resource intake. These two possibilities are non-exclusive and could be acting in simultaneously.

The cause of the increase in body size with altitude is actually being researched, and three possible explanations have been elucidated so far. To begin with, evidence has already been gathered indicating increasing resource availability and lizards' growth rate in the uppermost stations (unpublished data). There is also evidence that big lizards have increased thermoregulatory efficiency, as they are able to retain body heat longer (Zamora-Camacho et al. 2012a). Thus, big size could be selected in the upper, colder stations. To end with, survival rates could be higher (as found in other ectotherms, Zhang & Lu 2012). There is already evidence of decreased metabolic stress in high altitude populations (Reguera et al. 2012), and predation is probably lower, however this has not yet been confirmed. Thus, body size definitively appears to be controlled by environmental factors such as resource availability and temperature, however it is also plausible decreased predation rates could have an influence. As explained before, lower predation rates can result in an increased growth rate and survival, thus favouring the presence of large individuals in the population.

Reproductive status also proved to have an influence over lizards' back Lightness and Chroma, as well as overall colour difference variables. Gravid females display a lighter and more saturated colouration and present greater difference in colouration with ground and vegetation than males and non-gravid females. This result is counterintuitive as we would expect any shift in colouration associated to females' gravid state to increase crypsis. During pregnancy, females' weight and size increases reducing their mobility (e.g. Shine 1980). Hence, a cryptic strategy should be favoured instead of a fleeing one in response to predation (Bauwens & Thoen 1981). Also, clearer colourations would imply a reduction in thermoregulatory efficiency, which is also counterintuitive. If this selective pressure was the cause of the colouration shift, we would expect gravid females to be darker. This would increase their thermoregulatory efficiency compensating for the over-costs of pregnancy and reducing their exposure time to predators, in turn also compensating for decreased mobility.

The only explanation remaining (to our knowledge) that could account for gravid females' colouration being clearer and less saturated would be a shift in resource allocation during pregnancy, as pigment production implies high metabolic costs (e.g. Talloen et al. 2004; True 2003) and the synthesis of melanins is traded with immunocompetence (Moreno & Møller 2006). This hypothesis is consistent and would explain the fact that no differences in sprint speed were found between gravid and non-gravid females in these populations, also against what was expected (Zamora-Camacho et al. 2012b). Apparently, by maintaining sprint speed (fleeing strategy) gravid females are compensating for a forced (metabolically induced) reduction in colour matching.

Colour of *P. algirus* showed the lowest difference with its main basking sites, ground and rocks. In this case differences were lower for rocks than for ground, supporting the hypothesis that the former are also selected by this species for basking. Interestingly, males' colouration was less different form rocks than females', probably due to differential behaviour between sexes. During the reproductive season, males become more active (increasing their exposure to predation) and tend to select rocks as basking sites as they often provide a strategic position for territory guarding and courtship (Díaz 1993).

Lastly, correlations revealed tail loss frequency increased with male frequency along the gradient. This is consistent with males having higher predation rates (Medel et al. 1988) but could also be interpreted as tail loss being caused by intraspecific competition. Males often have agonistic encounters during the reproductive season in order to dispute territory or females (Díaz 1993; Salvador et al. 1996). During this encounters males often bite each other's tails sometimes triggering autotomy.

Tail loss frequency was also related to lizards' dorsal colouration. Lizards with autotomized tails were more yellow than those with regenerated and complete tails (lower Hue angle). One explanation for this would be that more yellow individuals are less cryptic, and thus, have increased predation rates. Nevertheless, this does not seem likely as no effect over colour difference variables was detected as a consequence of this variation; and regenerated and complete tails had similar Hue angles. Hence, this differential colouration seems to have no impact on crypsis. Most likely, changes in resource allocation or redirected vascularity during regeneration are responsible for this slight colour shift, however further research would be required to determine it.

6.- CONCLUSIONS

In conclusion, the degree of crypsis of *P. algirus* varied along an altitudinal gradient in Sierra Nevada. Colour matching was mainly directed to this lizards' preferred basking sites, ground and rocks, where they are most vulnerable to visual avian predators. The inter-population variance found in the degree of crypsis is probably caused by the variation in relative importance of differences in predator pressure or constrictions exerted by other selective pressures over colouration (thermoregulation, UV-protection). Results also reveal intra-population variation in the degree of crypsis, especially between individuals with different reproductive conditions (male, non-gravid female or gravid female). This variation is related to differential fleeing capacity, suggesting the existence of mixed strategies among the population: cryptic individuals with reduced fleeing capacity and more conspicuous individuals with increased fleeing capacity. Lastly, there are some evidences of costs associated to crypsis. Gravid females were less cryptic, probably as a consequence of a shift in resource allocation. Reproduction costs can limit an organisms' capacity to produce pigments (metabolically expensive), and hence to match substrate colouration.

7.- ACKNOWLEDGEMENTS

This study couldn’t have been carried out without the invaluable support, orientation and collaboration of my research directors Dr. Gregorio Moreno Rueda, Senda Reguera Panizo and Francisco Javier Zamora Camacho. In especial, PhD student Senda Reguera Panizo coached me patiently and diligently along the whole process and offered indispensable moral support. Also I am thankful to Elena Melero Martínez for her generous collaboration during the sampling campaign. The study has been economically supported by the Ministerio de Ciencia e Innovación (project CGL2009-13185).

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