The lizard *Psammodromus algirus* (Squamata: Lacertidae) is darker at high altitudes

SENDA REGUERA*, FRANCISCO J. ZAMORA-CAMACHO and GREGORIO MORENO-RUEDA

Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain

Altitudinal gradients offer a good opportunity to study organisms' adaptations to clinal environmental variables. Regarding altitude, the most influential variables on organisms are temperature and ultraviolet (UV) solar radiation, the first decreasing and the second increasing with altitude. Both variables affect ectotherms' biology, as ectotherms depend on environmental temperature for thermoregulation, frequently being heliotherms. Here, we studied dorsal coloration in the lizard *Psammodromus algirus* (Linnaeus, 1758) along a wide altitudinal gradient (2200 m) in Sierra Nevada (south-east Spain). We hypothesize that the skin will be darker with altitude, i.e. in environments with lower temperatures and higher UV radiation intensity. Results show that individual dorsal colorations became darker at high altitude. We propose two non-mutually exclusive explanations for this result: (1) darker dorsal surface would favour faster warming at high altitudes, where temperature is lower, and (2) darker dorsal surface would protect against UV radiation, stronger at high altitudes. We found significant relationships between both temperature and UV radiation and population dorsal darkness, giving mixed support for the two explanations. Moreover, dorsal hue was positively correlated with substrate hue, suggesting that hue evolved to maximize crypsis. Our study therefore suggests that geographical variation in dorsal coloration in this lizard is adaptive, and darkness coloration might have evolved in response to adverse conditions (low temperature and high UV radiation) at high altitudes.   © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **112**, 132–141.

ADDITIONAL KEYWORDS: altitudinal gradient – body colour – darkened coloration – thermal melanism – ultraviolet radiation.

INTRODUCTION

The environment gradually varies with altitude (Körner, 2007) and, accordingly, 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). In fact, animals may show adaptive variation in coloration in relation to elevation. For example, darker individuals are expected at higher elevations, where temperatures are lower, a pattern that may be explained by the 'thermal melanism hypothesis' (Clusella-Trullas, van Wyk & Spotila, 2007). This hypothesis states that, compared with light individuals, darker individuals have an advantage under low-temperature conditions, because they heat up faster at a given level of solar radiation (Watt, 1969; Clusella-Trullas *et al.*, 2007, 2008). Heating up faster is important for ectotherms, especially for reptiles, which depend largely on solar radiation for thermoregulation (Vitt & Caldwell, 2009). Populations distributed along an altitudinal range face temperature gradients, which provide a good opportunity to study the evolution of body coloration and its relationship to thermoregulation (Clusella-Trullas *et al.*, 2007).

A second hypothesis, non-mutually exclusive with the first, that could explain why organisms darken with altitude is based on the increasing UV radiation intensity with elevation ('protection against UV damage hypothesis', Porter & Norris, 1969). Most ectothermic organisms (in particular, heliotherms) rely on solar radiation to thermoregulate, but this radiation may have harmful effects at high altitude, where it is stronger. Moreover, given that thermal

*Corresponding author. E-mail: sreguera@ugr.es

energy availability is lower at higher altitudes, alpine reptiles, which furthermore spend more time sunbathing (Carrascal *et al.*, 1992; Martín & López, 1999; Gvoždík, 2002), would spend more time exposed to the harmful effects of solar radiation. High levels of UV radiation produce damage to DNA (Ravanat, Douki & Cadet, 2001), increase egg and embryo mortality, and reduce larva growth and development in amphibians (Belden, Wildy & Blaustein, 2000; Lizana & Pedraza, 2008; Marquis, Miaud & Lena, 2008), and cause tissue damages and cellular oxidative stress (Chang & Zheng, 2003). Consequently, organisms show several strategies to protect themselves from the harmful effects of UV radiation, sun-screen factors being one of the most important. Sun-screen factors, such as carotenoid and melanin pigmentation (Zellmer, 1995; Hessen, 1996; Gunn, 1998), protect against UV radiation by absorbing it (Hofer & Mokri, 2000; Cope *et al.*, 2001). Accordingly, given that UV radiation increases with altitude because the atmosphere is thinner (Blumthaler, Ambach & Ellinger, 1997), we expect individuals to darken at higher elevations.

Ectotherms tend to be darker at colder latitudes (Vidal, Ortiz & Labra, 2007; Alho *et al.*, 2010). Regarding altitude, melanic morphs of arthropods are more frequent at high altitudes (Zellmer, 1995; Hessen, 1996; Rajpurohit, Parkash & Ramniwas, 2008; more examples in Clusella-Trullas *et al.*, 2007). In reptiles, darker *Cordylus* species occur more frequently in colder and foggy areas, where melanism is interpreted as an advantage for faster heating (Janse van Rensburg, Mouton & van Niekerk, 2009). Moreover, melanic *Cordylus* spp. heat up faster, as a consequence of their low reflectance (Clusella-Trullas, van Wyk & Spotila, 2009). *Sceloporus occidentalis* (Baird and Girard, 1852) lizards from populations sited at high altitudes show darker colorations (Leaché, Helmer & Moritz, 2010), and *Podarcis hispanica* (Steindachner, 1870) lizards inhabiting colder environments are darker than conspecifics in warmer sites (Gabirot *et al.*, 2013).

On the other hand, geographical variation of darkness in lizard coloration may be due to nonphysiological selective pressures. For example, pressure of predation selects for cryptic coloration, which may be darker depending on substrate colour (Thorpe & Brown, 1989; Husak *et al.*, 2006; Jambrich & Jandzik, 2012). In this sense, clinal variation in substrate coloration might explain different coloration in animals.

Here we studied the shifts in the dorsal coloration of a lizard, the large psammodromus (*Psammodromus algirus* Linnaeus, 1758), along a 2200-m altitudinal gradient in south-eastern Spain, as possible adaptation in a clinal variation of environmental conditions.

This species inhabits a wide gradient of altitudes and environmental conditions (Salvador, 2011). As an ectotherm, *P. algirus* depends closely on environmental temperatures to thermoregulate (Díaz, Iraeta & Monasterio, 2006). These circumstances make *P. algirus* a suitable organism for this study. *Psammodromus algirus* is distributed between 200–2600 m above sea level on the Sierra Nevada Mountain of south-east Spain. Here, environmental temperatures decrease strongly with altitude (Zamora-Camacho *et al.*, 2013), while UV radiation increases (Sola *et al.*, 2008). We hypothesize that, in this elevation gradient, lizards' dorsal coloration darkens with altitude, under selective pressures such as lower environmental temperatures (because darker surfaces heat up faster) and/or higher UV radiation levels (because darker surfaces best protect against the harmful effects of UV radiation). Alternatively, changes in dorsal coloration may be a consequence of variation in substrate colour owing to predation pressure, because a greater similarity between dorsal coloration and the surroundings implies less detectability by predators.

Therefore, the main goal of this study was to test the hypothesis that dorsal coloration varies with altitude in *P. algirus*. Here, we predict darker individuals at higher elevations. In a second step, we tentatively test the different hypotheses explaining the variation in dorsal coloration with altitude. We test these hypotheses by correlating the average dorsal coloration of lizards in our six study populations with average temperature, UV radiation and substrate colour.

MATERIAL AND METHODS
GENERAL METHODS

Psammodromus algirus is a lacertid lizard of 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 a.s.l.), where it is exposed to a wide range of environmental conditions (Salvador, 2011). Dorsal coloration spans from pale to dark brown, with two dorsolateral yellow lines and a third mid-vertebral line in eastern populations (Salvador, 2011). Ventral ground-coloration goes from white to grey and frequently with yellow pigmentation in the throat region that varies in extent and intensity (Carretero, 2002). In western populations, males show orange pigmentation around the gular region during the breeding season (Salvador, 2011), but not in eastern populations, where males only show an orange spot in the commissures (Carretero, 2002). Dorsal pattern does not have sexual dimorphism.

Sampling was performed on Sierra Nevada (south-east Spain 3°37′59.8698″W, 36°54′57.8318″N), between 300 and 2500 m a.s.l. We established six populations separated by approximately 500 m in altitude (300, 700, 1200, 1700, 2200, 2500 m a.s.l.; Fig. 1), chosen according to similarity in slope and vegetation structure (more details in Supporting Information, in Methods S1). In the Sierra Nevada, mean annual temperature is 17.6–3.5 °C, according to altitude (261–3471 m a.s.l., respectively; Fig. S1), and in the study area air environmental temperature during the activity period (March to September) of *P. algirus* differs 8 °C on average (mean ± SD) between the lowest (300 m a.s.l.; 25.0 ± 5.09 °C) and the highest populations (2500 m a.s.l.; 17.2 ± 4.87 °C; Zamora-Camacho *et al*., 2013). Relative irradiance increases with altitude on average 6–8% km^{-1} for UV-A radiation and 7–11% km^{-1} for UV-B (Sola *et al*., 2008). In addition, UV radiation (300 nm wavelength) values on average ranged from 1.28 ± 0.03 μW cm^{-2} nm^{-1} in lowlands to 4.80 ± 0.37 μW cm^{-2} nm^{-1} in highlands (see measurement methodology below).

During 2010–2013 we captured by hand 492 lizards (255 females and 237 males) during their activity season (March to October) in the six populations ($N_{(altitude)}$ = females/males; N_{300} = 48/60; N_{700} = 38/22; N_{1200} = 32/19; N_{1700} = 38/35; N_{2200} = 51/42; N_{2500} = 48/59). Individuals were transported to a laboratory facility where colour characters were measured with a spectrophotometer (Minolta CM-2600d/2500d). Colour measures had the three chromatic values of the L*C*H* colour space, based on the L*a*b* colour space of the Commission Internationale d'Eclairage (CIE), which describes all the colours visible for most of the diurnal terrestrial vertebrates (Montgomerie, 2006). The coordinate L* represents *Lightness*, from black (L* = 0) to white (L* = 100). We also considered *chroma* (colour saturation), C* = [(a*)^2 + (b*)^2]$^{1/2}$ [0 in the centre of the colour space (unsaturated) and increases according to the distance from the centre]; and hue angle, H* = arc-tangent(b*, a*) in radians (after transforming radians into grades, it defines coloration as 0° for red, 90° for yellow, 180° for green and 270° for blue). Measures were obtained from two points (3 mm in diameter) of the dorsal surface, one at pileus and other at middle back (avoiding yellow lines).

Furthermore, SVL was measured with a metal ruler (accuracy 1 mm), and lizards were weighed with an electronic balance (Model Radwag WTB200, accuracy 0.01 g). We characterized sex based mainly on femoral pore development (more conspicuous in
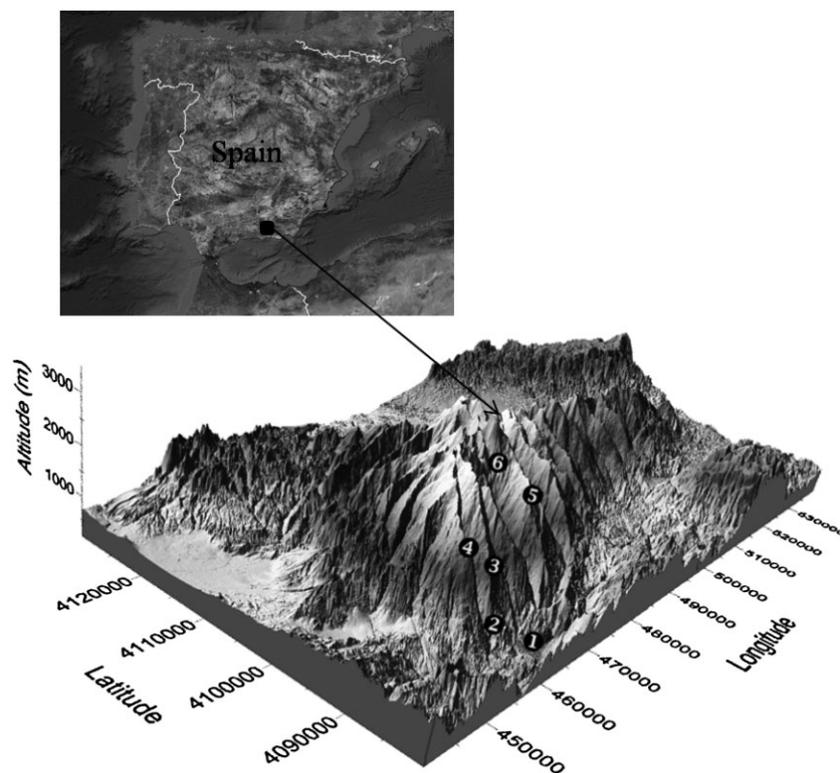
Figure 1. Location of Sierra Nevada Mountain in Spain and of the six populations in a three-dimensional map. Numbers indicate location of populations at 300 m a.s.l. (1), 700 m (2), 1200 m (3), 1700 m (4), 2200 m (5) and 2500 m (6).

males). To avoid the effect of ontogenetic shift in dorsal coloration (MANOVA test with colour traits as dependent variable and age category as factor; $F_{1,387} = 4.19$, $P < 0.001$), we only considered adult individuals in the analyses. We considered as adult those males with body size larger than the SVL of the smallest male with orange commissure and those females with body size larger than the SVL of the smallest gravid female. Both of are objective characters that reveal sexual maturity. These criteria were applied separately for each population, as body size (and thus minimal adult body size) varied with altitude (see Table S1).

Lizards were captured under permission (references GMN/GyB/JMIF and ENSN/JSG/JEGT/MCF) of the Junta de Andalucía and Parque Nacional de Sierra Nevada (Spanish Government) and were released at the capture site within 1 week. No lizard was damaged as a consequence of this study.

STATISTICAL ANALYSES

Variation in dorsal colour with altitude

To achieve the main goal of our study, we tested for differences in dorsal colour of lizards among altitudes. In this analysis, individual lizards were used as statistical units blocked by population (= altitude). We used lineal models (Quinn & Keough, 2002) run with R version 2.15.2 (R Development Core Team, 2012). Initially, six variables were considered for describing dorsal coloration: Pileus L*, Pileus C*, Pileus H*, Back L*, Back C* and Back H*. We generated a matrix of Pearson's partial correlations (controlling for altitude) to examine the relationships among the colour variables. The matrix of correlations showed that pileus and back coloration were highly correlated (all P values < 0.05; Table S2), so we included only back data in further analyses. Finally, we ran mixed effect linear models (LMMs) for each colour components (Zuur *et al.*, 2009): lightness, chroma and hue. We introduced in these analyses altitude (corresponding to the six populations sampled) and sex (male and female) as fixed factors, and body size as co-variable. Body size was estimated with a principal component analysis (PCA) in order to combine mass and SVL, as both were highly correlated ($r = 0.9$, $P < 0.001$, $N = 356$). In addition, we introduced month (from March to August) and year (from 2010 to 2013) as random factors, as we wanted to correct the possible variance they could be introducing in the model, but we were not interested in their effect on our dependent variables (colour components). We checked all interactions among independent variables, but only significant interactions remained in final models. For each model, we tested normality and homoscedasticity in residuals. Differences in coloration between

pairs of populations were tested with Tukey's 'Honest Significant Difference' post hoc test (Tukey HSD).

Relationship between population-level dorsal colour and environmental variables

To achieve the secondary goal of our study (a tentative test of three hypotheses explaining altitudinal variation in dorsal colour in this lizard), we tested the relationship among dorsal average coloration (at the level of population) with the characteristics of their population: environmental temperature, UV-B radiation, vegetation cover and substrate coloration. We measured these environmental variables in every population. (1) We recorded environmental temperature 1 m above the ground, under a shade, using a Hibok 14 thermometer (accuracy 0.1 °C) (more details in Zamora-Camacho *et al.*, 2013). (2) We measured UV-B radiation (μW cm^{-2} nm^{-1}) using a BIC compact four-channel radiometer (Biospherical Inc.). Radiation was measured twice a day at the beginning of August, every minute during a 10-min period. We used these data to predict radiation along the day, by using a polynomial model. Values of UV-B radiation were obtained by the 305-nm wavelength channel to pick lizards' activity period (11:00–18:00 h). (3) We measured vegetation cover at the middle of the growing season (spring). To measure the vegetation structure in each sampling station, we randomly set five transects 50 m long and recorded the presence or absence of vegetation (from grass-size to bush-size; see Methods S1) every metre. (4) We also measured ground coloration taking pictures of the substrate where lizards were captured and processing these pictures with Adobe Photoshop CS5 software (Pereira & Amat, 2010). After standardizing the pictures, we measured the average coloration in the L*a*b* colour space in 25 points (5 × 5 pixels), and estimated L*C*H* as described above.

Finally, using populations as statistical units, we correlated (Spearman correlations) mean values of lizards' dorsal coloration for each altitude with mean environmental temperature, mean UV-B radiation, percentage vegetation cover and mean values of substrate colour.

RESULTS

VARIATION IN DORSAL COLOUR WITH ALTITUDE

We found significant differences in all three dorsal colour components of *P. algirus* (lightness, chroma and hue angle) with altitude (Table 1, Fig. 2). Individuals from the two highest populations were darker (lower lightness values) than those in lower populations (Fig. 2). Dorsal coloration was less vivid (lower values of chroma) in the two highest populations

Table 1. Results of mixed effects linear models (ANOVA test; *F*-values) for each dorsal colour component

	d.f.	*F*-value	*P* value
Back lightness			
Altitude	5, 341	16.52	< 0.01
Body size	1, 341	8.90	< 0.01
Sex	1, 341	2.80	0.10
Altitude×Size	5, 341	3.38	0.01
Back chroma			
Altitude	5, 345	19.06	< 0.01
Body size	1, 345	0.64	0.42
Sex	1, 345	11.46	< 0.01
Back hue angle			
Altitude	5, 345	8.77	< 0.01
Body size	1, 345	0.01	0.91
Sex	1, 345	2.65	0.10

Colour components were included as dependent variables (lightness, chroma, hue angle), altitude and sex as fixed factors, and body size as covariate. The linear model was corrected for random factors including year and month. Only significant interactions are included.

(Fig. 2). Individuals were redder (lower values of hue angle) at 1700 m and especially at 2200 and 2500 m a.s.l. than at 300–1200 m a.s.l. Post hoc Tukey HSD tests revealed significant differences between both the 2200 and the 2500 m populations and the remaining populations for the three colour components measured (Table S3). Sexual differences were found only for chroma (Table 1). Males had more vivid dorsal coloration (mean ± SE; females = 12.26 ± 0.18, males = 13.03 ± 0.21). Finally, body size was an important predictor for lightness, even after controlling for altitude (Table 1). Larger individuals had higher values of lightness (β = 2.38). Nevertheless, we found a significant interaction between altitude and body size for dorsal lightness, given that the positive correlation between dorsal lightness and body size was found only in the populations at 300, 700 and 1700 m a.s.l. (Fig. 3).

RELATIONSHIP BETWEEN POPULATION-LEVEL DORSAL
COLOUR AND ENVIRONMENTAL VARIABLES

Temperature was negatively correlated with altitude, whereas UV radiation was positively correlated (Table 2; see mean population values of variables in Table S4). There was no significant correlation between altitude and vegetation cover (Table 2). Regarding substrate coloration, hue angle and lightness significantly covaried with altitude, substrate being darker as altitude increased (Table 2). Mean dorsal lightness of lizards decreased with altitude, but not significantly (Table 2). Moreover, lightness increased significantly with mean values of environmental temperature and decreased with UV radiation and vegetation cover (Table 2). Dorsal lightness did not covary with substrate lightness (Table 2). Mean values of dorsal chroma decreased significantly with altitude. Moreover, chroma increased with temperature and decreased with UV radiation (Table 2). Dorsal hue behaved similarly, decreasing significantly with altitude, increasing with temperature and decreasing with UV radiation (Table 2). Dorsal hue also was positively correlated with substrate hue (Table 2).

DISCUSSION

Psammodromus algirus showed dorsal colour variation in the altitudinal gradient examined in this study. Dorsal surface was darker at the highest altitudes (over 2000 m a.s.l.) and had less vivid and redder coloration. In addition, for a given altitude, larger individuals showed lighter coloration, especially at low altitudes. There were some differences between sexes, males having more vivid coloration.

Darker dorsal coloration at higher altitude has been observed in other animals (see Introduction). The 'thermal melanism hypothesis' states that darker coloration provides benefits in terms of body warming (Clusella-Trullas *et al*., 2007). For instance, in *Chamaeleo* spp. and *Sceloporus* spp., when temperature is low, pigments are dispersed through the entire cytoplasm, darkening the skin and improving thermoregulation (Walton & Bennett, 1993; Sherbrooke, Castrucci & Hadley, 1994). Consequently, darker individuals have thermal advantages under cold conditions, implying shorter periods of thermoregulation and increasing time available for other activities, which increases success in feeding opportunities, mates, defending territories and in escaping from predators (Clusella-Trullas *et al*., 2007). This advantage would make darker individuals in cold environments (e.g. in alpine habitats) larger and show better body condition than lighter individuals of the same population (Luiselli, 1993, 1995). Our results are consistent with this hypothesis, as populations where individuals showed darker skin (lower lightness values) were located at altitudes with the lowest temperatures. However, studies in a variety of lizard species on the relationship between coloration and warming rates have found mixed results, some failing to find a relationship (e.g. Herczeg, Török & Korsós, 2007), while other found that melanic lizards heated up faster than lighter lizards (e.g. Clusella-Trullas *et al*., 2009).

An alternative, non-exclusive, hypothesis is that dorsal darkness results from the selective pressure of UV radiation. UV radiation is more severe at higher elevations (Sola *et al*., 2008) and may produce damage
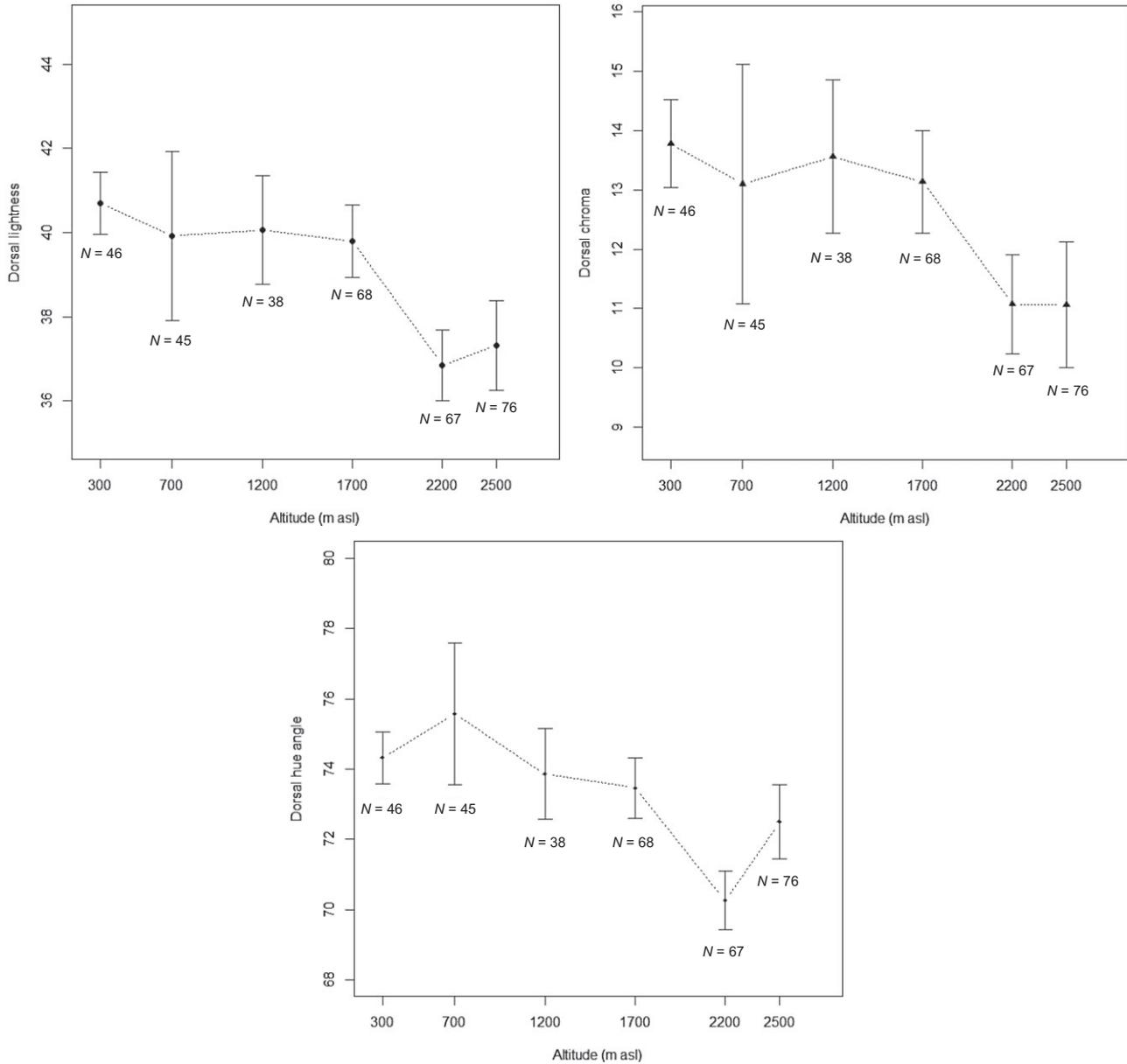
Figure 2. Mean values and standard error bars of dorsal coloration components (lightness, chroma and hue angle) of *Psammodromus algirus* in six populations (altitude; m.a.s.l.) along an elevation gradient in Sierra Nevada (south-east Spain). Sample size (*N*) is given below the mean value.

at different levels (see Introduction). In fact, higher reflectance of the skin is common in desert lizards (Porter & Norris, 1969). Accordingly, correlation between average skin lightness at the level of population and UV radiation intensity was significantly negative, darker individuals (low values of lightness) being found where UV radiation was highest, at high altitudes. The negative correlation between plant cover and dorsal coloration that we found may help to discern between the 'thermal melanism hypothesis' and the 'protection against UV radiation hypothesis'.

The 'thermal melanism hypothesis' would predict a positive correlation between covering and dorsal coloration, as in zones with higher plant cover, lizards face a cooler environment (Iraeta *et al.*, 2010), and would need to heat up faster, darker lizards therefore having an advantage. On the other hand, the 'protection against UV radiation hypothesis' would predict a negative correlation between plant cover and dorsal coloration, given that in more open zones lizards would be more exposed to radiation and darker lizards would have an advantage. Therefore, we
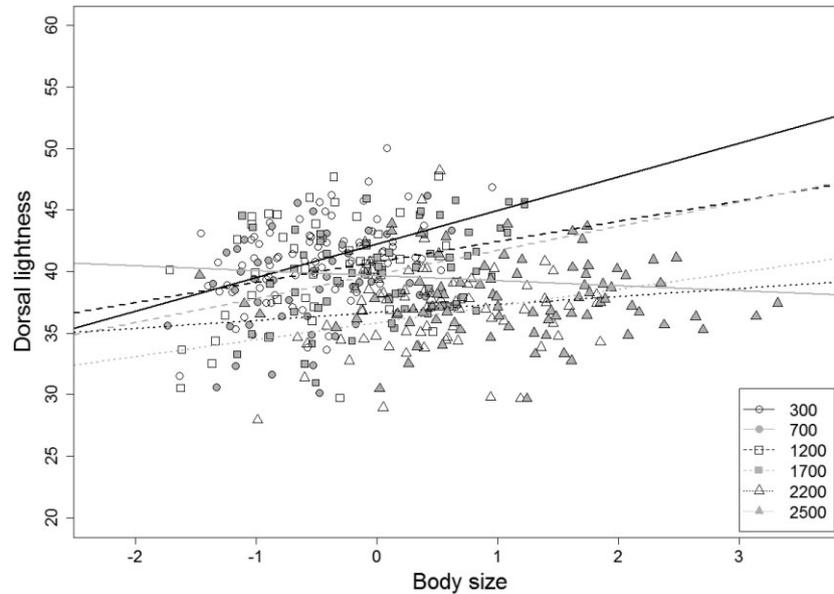
Figure 3. Dorsal lightness variation with body size (PCA factor of mass and snout–vent length) for each of six populations (i.e. altitude in m a.s.l.). Regression lines are represented for each altitude with lightness as dependent and body size as independent variable.

Table 2. Correlation (Spearman's correlation) matrix for mean values of components of lizards' dorsal colour [lightness (L*), chroma (C*) and hue angle (H*)], environmental temperature (T_e, in °C) during the active season, ultraviolet B radiation (UV-B, in µW cm^{-2} nm^{-1}), percentage vegetation cover (%VC) and substrate coloration for each altitude (corresponding to the studied populations)

	Altitude	Back L*	Back C*	Back H*	T_e	UV	%VC	Substrate L*	Substrate C*
Back L*	−0.77								
	0.07								
Back C*	**−0.94**	**0.89**							
	< 0.01	**0.02**							
Back H*	**−0.94**	**0.83**	**0.89**						
	< 0.01	**0.04**	**0.02**						
T_e	**−0.94**	**0.83**	**0.89**	**1.00**					
	< 0.01	**0.04**	**0.02**	**< 0.01**					
UV	**0.94**	**−0.83**	**−0.89**	**−1.00**	**−1.00**				
	< 0.01	**0.04**	**0.02**	**< 0.01**	**< 0.01**				
%VC	0.43	**−0.83**	−0.60	−0.60	−0.60	0.60			
	0.40	**0.04**	0.21	0.21	0.21	0.21			
Substrate L*	**−0.83**	0.54	0.77	0.77	0.77	−0.77	−0.14		
	0.04	0.27	0.07	0.07	0.07	0.07	0.79		
Substrate C*	0.77	−0.20	−0.60	−0.60	−0.60	0.60	−0.14	−0.71	
	0.07	0.70	0.21	0.21	0.21	0.21	0.79	0.11	
Substrate H*	**−0.94**	0.60	**0.83**	**0.89**	**0.89**	**−0.89**	−0.20	**0.94**	**−0.83**
	< 0.01	0.21	**0.04**	**0.02**	**0.02**	**0.02**	0.70	**< 0.01**	**0.04**

Sample size was six populations. *P* values are given below the correlation coefficients. Significant values are shown in bold type.

suggest that the negative correlation between plant cover and dorsal coloration tentatively gives support to the 'protection against UV radiation hypothesis' rather than the 'thermal melanism hypothesis'.

Other possible explanations for the altitudinal variation in dorsal darkness may be ruled out. First, in other lizards, predation selects for darker colorations that are more similar to substrate colour (Brown & Thorpe, 1991; Vidal *et al.*, 2007). Although substrate darkened with altitude, dorsal lightness of lizards' skin and lightness of substrate colour were not correlated, and thus this explanation was not supported. Second, melanin has been related to improved immunocompetence and higher resistance against parasites and pathogens in different taxa (Wilson *et al.*, 2001; Burtt & Ichida, 2004; Moreno & Møller, 2006). We have no data on how disease pressure varies with altitude in our study system, but preliminary analyses of immune response, measuring swelling foot pads after phytohaemagglutinin injection (a standard method to estimate cellular immune response, Smits, Bortolotti & Tella, 1999), suggest that immune response does not vary with altitude ($F_{5,96} = 1.064$, $P = 0.39$; our unpubl. data). And third, dark coloration may be a by-product of increased aggressiveness due to high population density, as frequently occurs in island lizard populations (Raia *et al.*, 2010). Nevertheless, we can discard this possibility in our study population, as the highest densities were found in populations at mid altitudes (1200 and 1700 m a.s.l.; Zamora-Camacho *et al.*, 2013), and there were no differences in coloration between mid and low altitudes (where the least dense populations occur).

We observed slight sexual dimorphism in dorsal coloration. Males had slightly more vivid colour than females. These results could be explained by the relationship of melanins and carotenoids with status and aggressiveness (Badyaev & Hill, 2000). On the other hand, note that males are more active than females and they are more exposed to sunlight (Díaz, 1993). Therefore, another possibility is that males accumulate more pigments (more saturated coloration) to protect them from radiation, or to heat up faster than females, and therefore to initiate activity earlier.

Chromatic components of the colour (chroma and hue) also varied with altitude. We tested whether differences in substrate colour acted as selective pressures favouring the noted variation (Macedonia, Echternacht & Walguarnery, 2003). Substrate coloration varied significantly with altitude, and lizard hue was positively correlated with substrate hue among populations. This finding suggests strongly that predation selects for lizards with dorsal colour similar to substrate colour. Therefore, it seems that predation selects for dorsal hue, while temperature and/or UV radiation selects for dorsal lightness in our study population.

In conclusion, this study shows that dorsal coloration shifts in an altitudinal gradient in the *P. algirus*, individuals from the highland populations being darker than those from mid- and low-altitude populations. We proposed three possible but non-exclusive hypotheses for this adaptation: the 'thermal melanism hypothesis' (Clusella-Trullas *et al.*, 2007), the 'protection against UV radiation hypothesis' (Porter & Norris, 1969) and the 'cryptic-coloration hypothesis'. Our findings give more support to the 'protection against UV radiation hypothesis', although the 'thermal melanism hypothesis' cannot be convincingly ruled out. On the other hand, the 'cryptic-coloration hypothesis' did not explain why lizards are darker at high altitudes, but this hypothesis explained variation in dorsal hue with altitude. In short, we suggest that *P. algirus* is adapted to a wide range of factors and one of these results in a darker dorsal colour at high altitude on a Mediterranean mountain.

REFERENCES

Alho JS, Herczeg G, Söderman F, Laurila A, Jönsson KI, Merilä J. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evolutionary Biology* **10:** 317.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57:** 1151–1163.

Badyaev AV, Hill GE. 2000. Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. *Biological Journal of the Linnean Society* **69:** 153–172.

Belden LK, Wildy EL, Blaustein AR. 2000. Growth, survival and behaviour of larval long-toed salamanders (*Ambystoma macrodactylum*) exposed to ambient levels of UV-B radiation. *Journal of Zoology* **251:** 473–479.

Blackburn TM, Ruggiero A. 2001. Latitude, elevation and body mass variation in Andean passerine birds. *Global Ecology and Biogeography* **10:** 245–259.

Blumthaler M, Ambach W, Ellinger R. 1997. Increase in solar UV radiation with altitude. *Journal of Photochemistry and Photobiology* **39:** 130–134.

Brown RP, Thorpe RS. 1991. Within-island microgeographic variation in the colour pattern of the skink, *Chalcides sexlineatus*: pattern and cause. *Journal of Evolutionary Biology* **4:** 557–574.

Burtt EH, Ichida JM. 2004. Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. *Condor* **106:** 681–686.

Carrascal LM, López P, Martín J, Salvador A. 1992. Basking and antipredator behaviour in a high altitude lizard: implications of heat-exchange rate. *Ethology* **92:** 143–154.

Carretero MA. 2002. Sources of colour pattern variation in Mediterranean *Psammodromus algirus*. *Netherlands Journal of Zoology* **52:** 43–60.

Chang C, Zheng R. 2003. Effects of ultraviolet B on epidermal morphology, shedding, lipid peroxide, and antioxidant enzymes in Cope's rat snake (*Elaphe taeniura*). *Journal of Photochemistry and Photobiology B: Biology* **72:** 79–85.

Clusella-Trullas S, Terblanche JS, Blackburn TM, Chown SL. 2008. Testing the thermal melanism hypothesis: a macrophysiological approach. *Functional Ecology* **22:** 232–238.

Clusella-Trullas S, van Wyk J, Spotila J. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology* **32:** 235–245.

Clusella-Trullas S, van Wyk JH, Spotila JR. 2009. Thermal benefits of melanism in cordylid lizards: a theoretical and field test. *Ecology* **90:** 2297–2312.

Cope RB, Fabacher DL, Lieske C, Miller CA. 2001. Resistance of a lizard (the green anole, *Anolis carolinensis*; Polychridae) to ultraviolet radiation-induced immunosuppression. *Photochemistry and Photobiology* **74:** 46–54.

Díaz JA. 1993. Breeding coloration, mating opportunities, activity, and survival in the lacertid lizard *Psammodromus algirus*. *Canadian Journal of Zoology* **71:** 1104–1110.

Díaz JA, Iraeta P, Monasterio C. 2006. Seasonality provokes a shift of thermal preferences in a temperate lizard, but altitude does not. *Journal of Thermal Biology* **31:** 237–242.

Gabirot M, Balleri A, López P, Martín J. 2013. Differences in thermal biology between two morphologically distinct populations of Iberian wall lizards inhabiting different environments. *Annales Zoologici Fennici* **50:** 225–236.

Gunn A. 1998. The determination of larval phase coloration in the African armyworm, *Spodoptera exempta* and its consequences for thermoregulation and protection from UV light. *Entomologia Experimentalis et Applicata* **86:** 125–133.

Gvoždík L. 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. *Canadian Journal of Zoology* **80:** 479–492.

Herczeg G, Török J, Korsós Z. 2007. Size-dependent heating rates determine the spatial and temporal distribution of small-bodied lizards. *Amphibia-Reptilia* **28:** 347–356.

Hessen DO. 1996. Competitive trade-off strategies in Arctic *Daphnia* linked to melanism and UV-B stress. *Polar Biology* **16:** 573–579.

Hofer R, Mokri C. 2000. Photoprotection in tadpoles of the common frog, *Rana temporaria*. *Journal of Photochemistry and Photobiology B: Biology* **59:** 48–53.

Husak JF, Macedonia JM, Fox SF, Sauceda RC. 2006. Predation cost of conspicuous male coloration in collared lizards (*Crotaphytus collaris*): an experimental test using clay-covered model lizards. *Ethology* **112:** 572–580.

Iraeta P, Salvador A, Monasterio C, Díaz JA. 2010. Effects of gravidity on the locomotor performance and escape behaviour of two lizard populations: the importance of habitat structure. *Behaviour* **147:** 133–150.

Jambrich A, Jandzik D. 2012. Melanism in the topotypic population of the Pannonian subspecies of the common lizard, *Zootoca vivipara pannonica* (Reptilia: Lacertidae). *Herpetology Notes* **5:** 219–221.

Janse van Rensburg DA, Mouton PleFN, van Niekerk A. 2009. Why cordylid lizards are black at the south-western tip of Africa. *Journal of Zoology* **278:** 333–341.

Körner C. 2007. The use of 'altitude' in ecological research. *Trends in Ecology & Evolution* **22:** 569–574.

Leaché AD, Helmer D-S, Moritz C. 2010. Phenotypic evolution in high-elevation populations of western fence lizards (*Sceloporus occidentalis*) in the Sierra Nevada Mountains. *Biological Journal of the Linnean Society* **100:** 630–641.

Lizana M, Pedraza EM. 2008. The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. *Conservation Biology* **12:** 703–707.

Luiselli L. 1993. The ecological role of color polymorphism in male adders, *Vipera berus*: testing the hypotheses. *Revue d'Ecologie-La Terre et la Vie* **48:** 49–56.

Luiselli L. 1995. Body size, sexual size dimorphism and reproduction in different colour morphs in a population of Western whip snakes, *Coluber viridiflavus*. *Revue d'Ecologie-La Terre et la Vie* **50:** 365–376.

Macedonia JM, Echternacht AC, Walguarnery JW. 2003. Color variation, habitat light, and background contrast in *Anolis carolinensis* along a geographical transect in Florida. *Journal of Herpetology* **37:** 467–478.

Marquis O, Miaud C, Lena JP. 2008. Developmental responses to UV-B radiation in common frog *Rana temporaria* embryos from along an altitudinal gradient. *Population Ecology* **50:** 123–130.

Martín J, López P. 1999. When to come out from a refuge: risk-sensitive and state-dependent decisions in an alpine lizard. *Behavioral Ecology* **10:** 487–492.

Montgomerie R. 2006. Analyzing colors. In: Hill GE, McGraw

KJ, eds. *Bird coloration: mechanisms and measurements*. Cambridge, MA: Harvard University Press, 90–140.

Moreno J, Møller AP. 2006. Are melanin ornaments signals of antioxidant and immune capacity in birds? *Acta Zoologica Sinica* **52:** 202–208.

Pereira AI, Amat JA. 2010. Nesting of the double-striped thick-knee (*Burhinus bistriatus*) in a hot environment. *Ornitología Neotropical* **21:** 149–154.

Porter WP, Norris KS. 1969. Lizard reflectivity change and its effect on light transmission through body wall. *Science* **163:** 482–484.

Quinn GP, Keough MJ. 2002. *Experimental design and data analysis for biologists*. New York: Cambridge University Press.

Raia P, Guarino FM, Turano M, Polese G, Rippa D, Carotenuto F, Monti DM, Cardi M, Fulgione D. 2010. The blue lizard spandrel and the island syndrome. *BMC Evolutionary Biology* **10:** 289.

Rajpurohit S, Parkash R, Ramniwas S. 2008. Body melanization and its adaptive role in thermoregulation and tolerance against desiccating conditions in drosophilids. *Entomological Research* **38:** 49–60.

Ravanat JL, Douki T, Cadet J. 2001. Direct and indirect effects of UV radiation on DNA and its components. *Journal of Photochemistry and Photobiology B, Biology* **63:** 88–102.

R Development Core Team. 2012. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Salvador A. 2011. *Lagartija colilarga – Psammodromus algirus* (Linnaeus, 1758). In: Salvador A, Marco A, eds. *Enciclopedia virtual de los vertebrados españoles*. Madrid: Museo Nacional de Ciencias Naturales (CSIC). Available at: http://www.vertebradosibericos.org/reptiles/psaalg.html

Sherbrooke WC, Castrucci AMdeL, Hadley ME. 1994. Temperature effects on in vitro skin darkening in the mountain spiny lizard, *Sceloporus jarrovi*: a thermoregulatory adaptation? *Physiological Zoology* **67:** 659–672.

Smits JE, Bortolotti GR, Tella JL. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13:** 567–572.

Sola Y, Lorente J, Campmany E, de Cabo X, Bech J, **Redaño A, Martínez-Lozano JA, Utrillas MP, Alados-Arboledas L, Olmo FJ, Díaz JP, Expósito FJ, Cachorro V, Sorribas M, Labajo A, Vilaplana JM, Silva AM, Aadosa J. 2008.** Altitude effect in UV radiation during the Evaluation of the Effects of Elevation and Aerosols on the Ultraviolet Radiation 2002 (VELETA-2002) field campaign. *Journal of Geophysical Research* **113:** 1–11.

Thorpe RS, Brown RP. 1989. Microgeographic variation in the colour pattern of the lizard *Gallotia gallotia* within the island of Tenerife: distribution, pattern and hypothesis testing. *Biological Journal of the Linnean Society* **38:** 303–322.

Vidal MA, Ortiz JC, Labra A. 2007. Sexual and geographic variation of color patterns in *Liolaemus tenuis* (Squamata, Liolaeminae). *Gayana* **71:** 27–33.

Vitt LJ, Caldwell JP. 2009. *Herpetology: an introductory biology of amphibians and reptiles*. London: Academic Press.

Walton BM, Bennett AF. 1993. Temperature-dependent color change in Kenyan chamaleons. *Physiological Zoology* **66:** 270–287.

Watt WB. 1969. Adaptive significance of pigment polymorphisns in *Colias* butterflies, II. Thermoregulation and photoperiodically controlled melanin variation in *Colias eurytheme*. *Zoology* **63:** 767–774.

Welter-Schultes FW. 2000. The pattern of geographical and altitudinal variation in the land snail *Albinaria idaea* from Crete (Gastropoda: Clausiliidae). *Biological Journal of the Linnean Society* **71:** 237–250.

Wilson K, Cotter SC, Reeson AF, Pell JK. 2001. Melanism and disease resistance in insects. *Ecology Letters* **4:** 637–649.

Zamora-Camacho FJ, Reguera S, Moreno-Rueda G, Pleguezuelos JM. 2013. Patterns of seasonal activity in a Mediterranean lizard along a 2200 m altitudinal gradient. *Journal of Thermal Biology* **38:** 64–69.

Zellmer ID. 1995. UV-B-tolerance of alpine and arctic *Daphnia*. *Hydrobiologia* **307:** 153–159.

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. *Mixed effects models and extensions in ecology with R*. New York: Springer.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Methods S1. Detailed description of the study area.
Figure S1. Altitude and mean values of precipitation and temperature in Sierra Nevada.
Figure S2. Three-dimensional map of Sierra Nevada, showing the location of the sampling plots (1–6 correspond to 300–2500 m a.s.l.). The location of Sierra Nevada in the Iberian Peninsula is also indicated.
Figure S3. Percentage of ground surface covered by vegetation in each altitude ($N = 253$ measures in every altitude).
Figure S4. Percentage of ground surface covered for each vegetation–height category in altitude ($N = 253$ measures in every altitude).
Table S1. Classification of age categories by altitude.
Table S2. Relationship between pileus and back coloration.
Table S3. Post-hoc analyses for the colour variation in altitude.
Table S4. Mean population values of variables.