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Coloration reflects skin pterin concentration in a red-tailed lizard

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ABSTRACT

When integumentary tissue pigments are contained in chromatophores, tissue color might not depend exclusively on the amount of pigment. Whether coloration does or does not reflect pigment concentration may be very significant for intraspecific communication, for example when pigment concentration provides fitness-related information. We studied the pigment responsible for the orange/red ventral tail coloring in a lacertid lizard species (*Acanthodactylus erythrurus*), and whether the color was related to skin pigment concentration. The pigment was identified as a pterin, a higher concentration of which resulted in darker, more red-saturated, redder (less orange) ventral tail skin color. The dorsal tail integument, even though it appears mostly gray to the naked eye, also contained pterins, and furthermore, the dorsal and ventral pterin concentrations were positively correlated. A possible explanation for these results is that pterins accumulate in the skin of the whole tail, even if only needed in the ventral part, but are concealed in the dorsal part. In this way, ventral orange/red coloration would accurately reflect pterin concentration, which provides the basis for a signaling function, while dorsal coloration would become less conspicuous as an anti-predatory mechanism.

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1. Introduction

Coloration exhibited by animals is, in most cases, the result of selective absorption, reflection and/or refraction of the light incident on the skin or other external structures such as hair or feathers (Fox, 1976). When selection of certain wavelengths is caused by the microstructure of the surface, as it happens with most blue and green hues in animals, it is known as structural coloration (Kinoshita et al., 2008). But when wavelength selection is caused by pigments, which are molecules that absorb part of the light spectrum, it is known as pigmentary coloration. The most common types of pigments in integumentary tissues are melanins (e.g. eumelanin and pheomelanin), responsible for black, gray and brown colors (Slominski et al., 2004), and carotenoids (e.g. lutein, astaxanthin and canthaxanthin), typically producing yellow, orange and red hues (Goodwin, 1984). However, many other types of pigments such as pterins, porphyrins, or psittacofulvins have been also found in animals (McGraw, 2006).

Pigmentary coloration plays a fundamental role in animal communication, both within and between species (Cott, 1940). In interspecific communication, these color signals are common subjects of study in predator–prey interactions due to aposematism and mimicry (Stevens, 2007). Coloration can also be useful to divert predator attacks towards non-vital body parts (Van Buskirk et al., 2004), or in association with some behaviors, inform the predator that it has been spotted and would waste time and energy if launching an attack (Alvarez, 1993). In intraspecific communication, pigmentary colors may signal a wide array of individual characteristics, both in sexual and non-sexual interactions (Senar, 2006; Blount and McGraw, 2008). For instance, coloration may play an important role in mate acquisition by signaling fertility or health status (Weiss, 2006; Pitcher et al., 2007; del Cerro et al., 2010), and thus making the bearer more sexually attractive (Deere et al., 2012). Coloration may also reflect aggressiveness or dominance, crucial traits when individuals compete for access to mates or to non-sexual resources such as territories or food (Senar, 2006). Communication between age classes is another important intraspecific function of color signals in many taxa. For example, juvenile coloration may reduce aggression from adults (VanderWerf and Freed, 2003) and affect parental investment (de Ayala et al., 2007).

The kind of pigment responsible for the color might have profound implications on the evolution of coloration as a signal, because different pigments usually have different properties. For example, while some pigments (e.g. carotenoids) cannot generally be synthesized de novo by animals (Britton, 1998) and might thus signal the ability to obtain resources, others (e.g. pterins) are synthesized within animal cells (Ziegler, 1965; Brown, 1985) and would not be as well suited to signaling that ability. Therefore, determining the pigment responsible for a particular color would be a good first step in studying the function and evolution of that color. Although some animal colors are usually caused by specific pigments (e.g. black is generally caused by eumelanin), the pigment responsible often cannot be inferred for

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other colors (McGraw et al., 2004). For instance, a similar red color (blood red according to Toral et al., 2008) in avian feathers can be caused by pheomelanin in the barn swallow (*Hirundo rustica*) (McGraw et al., 2004) or by carotenoids in the wallcreeper (*Tichodroma muraria*) (Bleiweiss, 2014). In another example, the bright yellow color of the iris is produced by carotenoids in the short-eared owl (*Asio flammeus*) and by pterins in the great horned owl (*Bubo virginianus*) (Oliphant, 1988).

In some integumentary tissues (e.g. feathers), pigments are embedded in proteins, and the intensity of the color is usually directly proportional to the amount of pigment (Saks et al., 2003; McGraw et al., 2005; Galvan et al., 2012). However, in some cases, the relationship between pigment concentration and color is not so obvious. Skin coloration in some animal taxa (e.g. reptiles, fish or cephalopods) is mainly due to pigment-containing cells called chromatophores (Grether et al., 2004; Bagnara and Matsumoto, 2007). The amount of pigment contained in the chromatophore does not necessarily determine the color, as it is demonstrated in animals that can change skin color quickly (e.g. squids; Hanlon et al., 1999). This color change may be due to dispersion or aggregation of the pigment in the cell (Logan et al., 2006) or to expansion or retraction of the chromatophore, with the consequent change in size and/or shape (Messenger, 2001). Even in animals that cannot change skin color quickly, all or part of the pigments might be concealed and not contribute to coloration. Moreover, tissue microstructure may alter pigmentary coloration dramatically. For example, green skin color in male panther chameleons (*Furcifer pardalis*) is the result of the interaction between yellow pigments and a layer of iridophores, cells that contain light-reflective guanine crystals (Teyssier et al., 2015). Animal excitation causes an increase in the distance among crystals and skin color change from green to yellow (Teyssier et al., 2015). Similarly, in other lizard species, reflectance of the layer of iridophores also contributes greatly to pigment-based coloration (e.g. Morrison et al., 1995; San-Jose et al., 2013). Given the different characteristics of chromatophores and background tissue (apart from pigment content) that affect pigmentary coloration, it is not surprising that several studies have found a weak (e.g. Weiss et al., 2012) or no (e.g. Garner et al., 2010; San-Jose et al., 2013) relationship between skin pigment concentration and coloration.

Whether coloration does or does not accurately reflects pigment concentration may have implications for signal honesty. Animal communication often occurs among individuals with conflicting interests, and mechanisms maintaining signal honesty are then required. For a signal to be reliable, its expression should entail fitness costs that are more affordable for high-quality than for low-quality individuals (Zahavi, 1975; Grafen, 1990). Color signals in general can be costly for different reasons (e.g. increased predation; Stuart-Fox et al., 2003), but pigment-based color signals in particular might also be costly because of the properties of the pigment. For example, when pigments have immune or antioxidant functions (e.g. carotenoids and pterins; McGraw, 2005), there is a trade-off between devoting pigments to become colorful or to the other physiological processes (including, for example, egg yolk formation in the case of reproductive females). In this case, only individuals with low immune and antioxidant requirements (i.e., healthy animals) will be able to use pigments mostly for coloration (Mougeot et al., 2010). It has been also suggested that pigment-based coloration might signal the efficiency of cellular respiration through the oxidation of pigments (Johnson and Hill, 2013).

Our study species was *Acanthodactylus erythrurus* (Schinz 1833), a North African/Southern European lizard species of the Lacertidae family. Juveniles of this species develop orange/red coloration on the rear part of their hind limbs and the ventral part of their tails (Fig. 1F) (Seva Román, 1982; Carretero and Llorente, 1993). The identity of the pigment responsible for this coloration is currently unknown. Juvenile males lose their orange/red color when approaching sexual maturity, whereas juvenile females retain it through adulthood (Seva Román, 1982). The orange/red coloration of adult females retained from the juvenile phase increases in intensity at the beginning of the reproductive season until they are gravid, when it is gradually lost and becomes light buff-gray, nearly white (Fig. 1D) (Cuervo and Belliure, 2013). In contrast, adult males show white coloration on the rear part of their hind limbs and the ventral part of their tails during the whole reproductive season (Fig. 1B) (Seva Román, 1982). Dorsal tail color is brownish gray with light and dark patches and stripes (Fig. 1H).

Orange/red coloration in most lizard species studied to date is caused by pterins (e.g. Ortiz et al., 1962; Ortiz and Maldonado, 1966; Weiss et al., 2012), although in some species it is caused by carotenoids (e.g. Hamilton et al., 2013). When both types of pigments are present, mainly pterins contribute to this color (Ortiz et al., 1963; Macedonia et al., 2000; Steffen and McGraw, 2009). Pheomelanin can also produce reddish colors in some animal taxa (e.g. in birds; Toral et al., 2008), but this kind of pigment has never been found in lizards or in any Squamata (the order including lizards and snakes). Pheomelanin has been found in a reptile species, namely a tortoise (Roulin et al., 2013), but Testudines (the order including tortoises and turtles) are not close relatives of lizards. In fact, lizards are phylogenetically as distant from Testudines as they are from birds (Wang et al., 2013).

The aims of this study were (1) to identify the pigment responsible for orange/red coloration and (2) to determine whether orange/red coloration reflects skin pigment concentration in *A. erythrurus*, a lizard species in which chromatophores are responsible for skin color.

2. Materials and methods

2.1. Field procedures

The study was carried out in May 2009 in Almería, south-eastern Spain. We captured lizards in Cabo de Gata-Níjar Natural Park (36°49′08″–36°50′13″ N, 2°16′59″–2°18′36″ W), in open coastal scrubland with sandy soils. A total of 30 individuals were captured with a noose at the end of a 2-m-long fishing pole and placed in individual cloth bags (23 × 28 cm) inside a cooler to avoid overheating. They were five adult males (white tail), six adult females (light buff-gray tail, as they were captured around the egg-laying period; Cuervo and Belliure, 2013) and 19 juveniles (orange/red tail). Juveniles could not be sexed because sexual dimorphism in juveniles of this species is not yet evident in spring of their first year. We then took all animals to the Finca Experimental La Hoya, a facility of the Arid Zones Experimental Station (EEZA-CSIC) in Almería city, about 20 km from the area where lizards were captured. In the laboratory, we measured and weighed the lizards and quantified tail color (see Color measurement). Immediately after color measurement, we detached the tail around 2 cm from the cloaca by gently pulling with the fingers. As this species has caudal autotomy (Belliure, 2009), the force needed to detach the tail was small and there was virtually no bleeding. A 1-cm-long piece of the proximal part of the detached tail was then cut with scissors and frozen at −70 °C until pigment analysis (see Pigment determination and quantification). All animals were released less than 24 h after capture in the same place where they had been captured. All of them behaved normally when released. We did not mark lizards individually, but unnoticed recaptures were not possible because only individuals with intact tail were included in the study. Individuals with regenerated tails were excluded because regenerated tails differ in coloration from original ones in many lizard species (e.g. Kwiatkowski, 2003; Ritzman et al., 2012), including *A. erythrurus* (personal observation), and this might affect the results.

2.2. Color measurement

We recorded reflectance spectra (325–700 nm) with an Ocean Optics USB2000 spectrophotometer (Ocean Optics, Inc., Dunedin, FL, USA) connected to a deuterium tungsten halogen light source (DT-MINI-2-GS). Reflectance was always measured with the coaxial

reflection probe (QR400-7-UV/BX) placed gently in contact with the skin (with no pressure applied) at a 45° angle. The measurements were referenced to standard white (WS-1) and dark (black electrical tape), which were calibrated before measuring each lizard. Data were processed with SpectraSuite (Ocean Optics, Inc., Dunedin, FL, USA) software. We measured reflectance five times for each individual on the ventral part of the tail around 2.5 cm from the cloaca. We took the five measurements in a row, and the probe was repositioned after each measurement. Reflectance spectra and photographs of the ventral part of the tail can be found in Fig. 1. We calculated three color parameters from reflectance data following standard procedures (Montgomerie, 2006): brightness (Σ reflectance 325–700 nm), red chroma (Σ reflectance 625–700 nm divided by Σ reflectance 325–700 nm), and hue (wavelength of maximum reflectance). The repeatability of measurements (Lessells and Boag, 1987) was estimated from the 30 lizards, and was high for brightness ($r = 0.906$) and red chroma ($r = 0.980$), but relatively low for hue ($r = 0.340$). In any case, within-individual variability of the three parameters was significantly less than among-individual variability (ANOVAs; $F_{29,120} \geq 3.57$, $p < 0.00001$), thus justifying the use of means for each individual in later analyses.

Dorsal tail color appeared to be mostly gray to the naked eye, so red pigments were not expected and dorsal tail color was not quantified in this study. However, dorsal tail skin turned out to contain red pigments in a subsample of juveniles (see Results), thus making

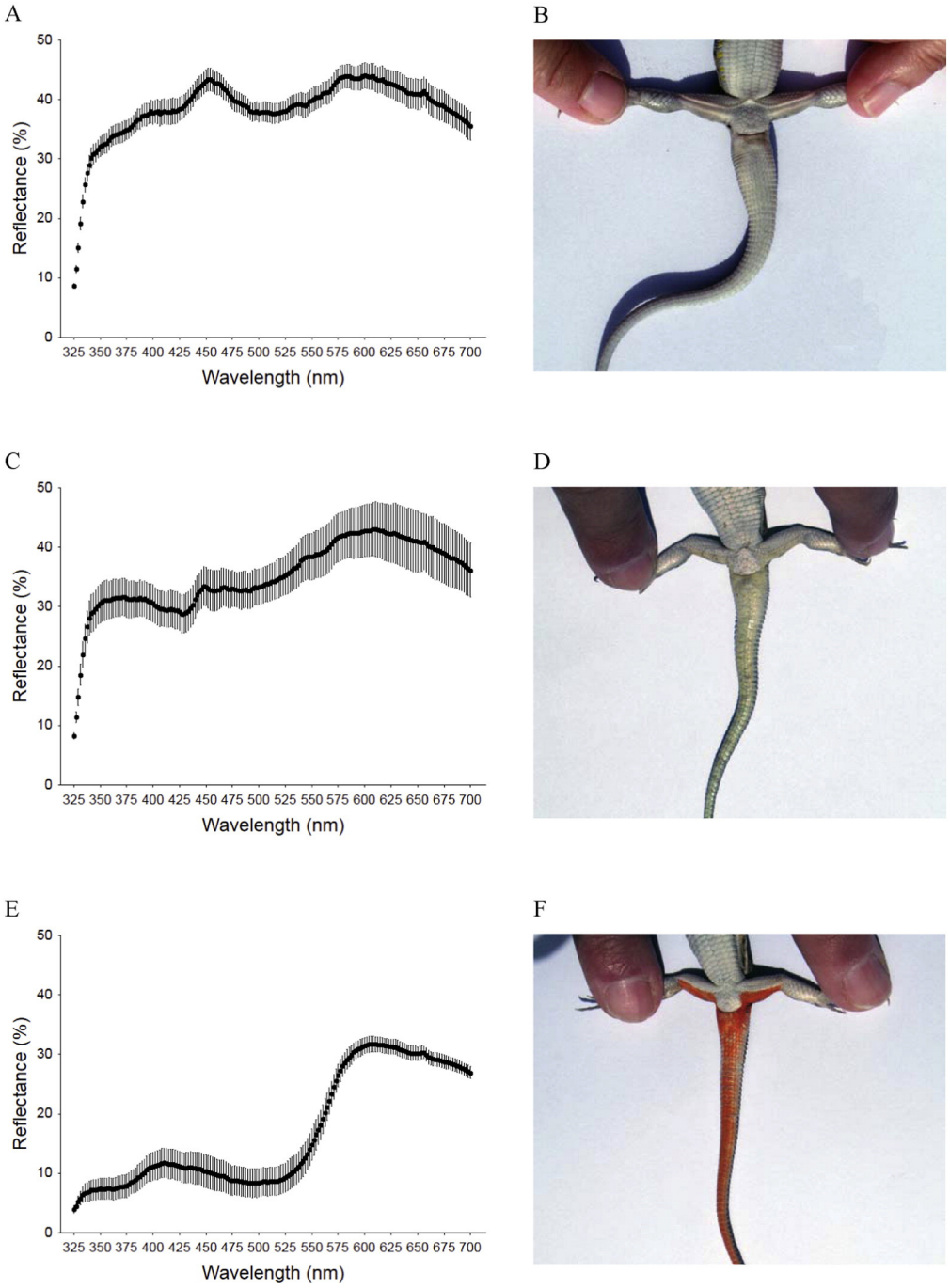

Fig. 1. Average (±SE) reflectance spectra and photographs of the ventral part of the tail in *Acanthodactylus erythrurus* lizards. (A, B) Adult males ($n = 5$). (C, D) Adult females ($n = 6$; reflectance was measured around the egg-laying period, when adult females had lost their red color). (E, F) Juveniles ($n = 19$). (G, H) Average (±SE) reflectance spectra and a photograph of the dorsal part of the tail in juveniles ($n = 12$) not included in this study are also shown for illustrative purposes.

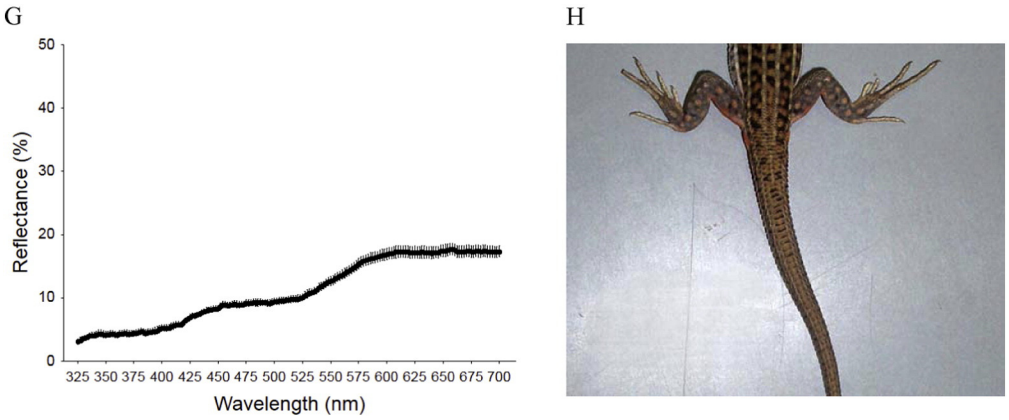

Fig. 1 (*continued*).

spectrophotometric measurements of the dorsal part of the tail interesting. Unfortunately, when the presence of red pigments in dorsal skin was discovered, it was impossible to recapture the same individuals for which pigment content had been determined. Even if they had been recaptured, their coloration might have changed because they would not be juveniles any more, and because they would have regenerated tails. Although we did not quantify dorsal tail color in this study, 12 juveniles were captured in 2011 for another study and reflectance of the dorsal part of the tail was measured using the method described above. Reflectance spectra and a photograph of the dorsal part of the tail in these lizards are shown in Fig. 1G and H for illustrative purposes only.

2.3. Pigment determination and quantification

As the pigment producing red coloration in *A. erythrurus* was unknown, we determined whether carotenoids, pterins or both were involved. Firstly, about 0.5 cm^2 of red skin was removed from the ventral area of a juvenile's tail and ground in a mixer mill in 3% NH_4OH. We transferred the ground material and solvent to a fresh tube, and added tert-butyl methyl ether (TBME) to partition carotenoids from pterins (Steffen and McGraw, 2007). The upper phase (TBME) and the lower phase (NH_4OH) were separated and analyzed by absorbance spectrophotometry in the 200–500 nm range. No carotenoid signal was apparent in the TBME phase, whereas a peak was detected around 350 nm in the ammonium phase, consistent with the presence of a (still unidentified) pterin. Secondly, we compared a juvenile red skin sample to a sample of fresh red *Drosophila melanogaster* eyes, which are known to contain drosopterins (Wilson and Jacobson, 1977). About one hundred wild-type *Drosophila* flies were euthanized by deep freezing and their eyes were removed manually using two pins under a stereomicroscope. We extracted both the lizard and *Drosophila* samples using Ethanol — 3% NH_3. The comparison was done using thin-layer chromatography (silica gel C-18) with a solvent system consisting of 1:1 isopropanol — 2% ammonium acetate. For both samples, matching lower spots were red-orange in color under UV light and were ascribed to a drosopterin. Later spectrophotometric analysis of the extracts showed a peak at 490 nm, again typical of drosopterins. The chromatographic techniques used here are described in more detail elsewhere (Negro et al., 2009).

When the nature of the red pigment had been determined, we estimated its concentrations in the samples ($n = 30$) by HPLC analysis at the Chemical Ecology Laboratory of Doñana Biological Station (EBD-CSIC; Seville, Spain) using a Waters 2695 separations module (Waters Corporation, Milford, MA, USA). In tail samples (5–7-mm-long cylinders), we separated the skin from the muscle, and then the red (or white) ventral skin from the dark dorsal skin. Every portion was weighed to the nearest 0.01 mg, treated with 750 μl of 1% NH_3, and sonicated until the skin became colorless. We then centrifuged the solvent at 13,000 rpm for 5 min, and kept 150 μl in a HPLC vial. Pterin was also extracted from dorsal dark skin and muscle of eight individuals (seven juveniles and one adult female) chosen randomly among the available samples. We analyzed pterin using a reversed-phase C18 column (Sunfire C18, Waters Corporation, Milford, MA, USA) and a precolumn of the same material with 5-μm particle size (Fukushima and Nixon, 1979). The eluent system was a gradient from CH_3COONH_4 (10 mM)–MeOH (97:3) to CH_3COONH_4 (10 mM)–MeOH (83.5:16.5), with a flow rate of 0.5 ml/min for 40 min. Injection volume was 30 μl. Data were acquired between 195 nm and 650 nm with a PDA 2998 multi-wavelength detector (Waters Corporation, Milford, MA, USA). Two peaks were found. The one we assumed to be drosopterin had a retention time of 20 min and maximum absorbance at around 490 nm. In fact, it was composed of several peaks very close to each other corresponding to different components of the drosopterin family (Kim et al., 2013). We integrated all these peaks together and considered them a single peak for calculation of pigment concentration. The second peak was located at 340 nm and could not be identified (it was not present in our pigment library), but pterins or any other pigment providing color can be excluded based on its retention time in the HPLC system and its absorption spectrum. We calculated pterin concentration from the HPLC area under the 490 nm peak, considering the mass of the skin or muscle (in mg) used for pigment extraction. HPLC areas were relative values with no specific units and, consequently, pterin concentrations were also relative values without units.

2.4. Statistical analyses

Ventral pigment concentration was Box-Cox transformed ($(\text{variable} + 3000)^{0.3}$) to achieve normality, but the three color parameters followed a normal distribution without transformation (Kolmogorov–Smirnov test for normality, $p > 0.10$). We tested the relationships between color parameters and ventral pigment concentration using linear regressions. These relationships were tested for all 30 lizards (three tests, one for each color parameter) and also for the subsample of juvenile lizards ($n = 19$; other three tests). We tested the relationships including dorsal or muscle pigment concentrations using Spearman rank-order correlations (non-parametric statistics) because of small sample size ($n = 8$). The relationships between color parameters and ventral pigment concentration were also tested including exclusively the subsample of individuals with information for dorsal pigment concentration ($n = 8$) and using Spearman rank-order correlations. We used the Wilcoxon matched-pairs test to compare pigment concentration between ventral and dorsal tail skin. All non-parametric statistics were performed on raw data. Some samples (see Supplemental Table 1) contained such small amounts of pigment that concentrations might not be reliable. Therefore, we repeated the

statistical analyses setting pigment concentrations in all these cases to zero, but results were qualitatively identical (see Supplemental Table 2). In this study we performed multiple statistical tests, and it is well known that the risk of incurring Type I error increases with the number of tests performed. To alleviate this problem, we used sequential Bonferroni correction (Rice, 1989), but with a 10% level of significance to decrease the risk of incurring Type II error (Chandler, 1995). The number of tests included in the correction was $k = 9$ (3 color parameters × 3 tail parts). In order to check the robustness of the results, most tests were repeated with different subsamples (e.g. including juveniles only) or with different values (e.g. setting very small pigment concentrations to zero), but these repetitions cannot be considered multiple testing. All statistical tests were two-tailed.

3. Results

We were able to demonstrate, using different techniques (spectrophotometry, thin-layer chromatography and HPLC), that carotenoids were absent in ventral tail skin of a juvenile *A. erythrurus*, and

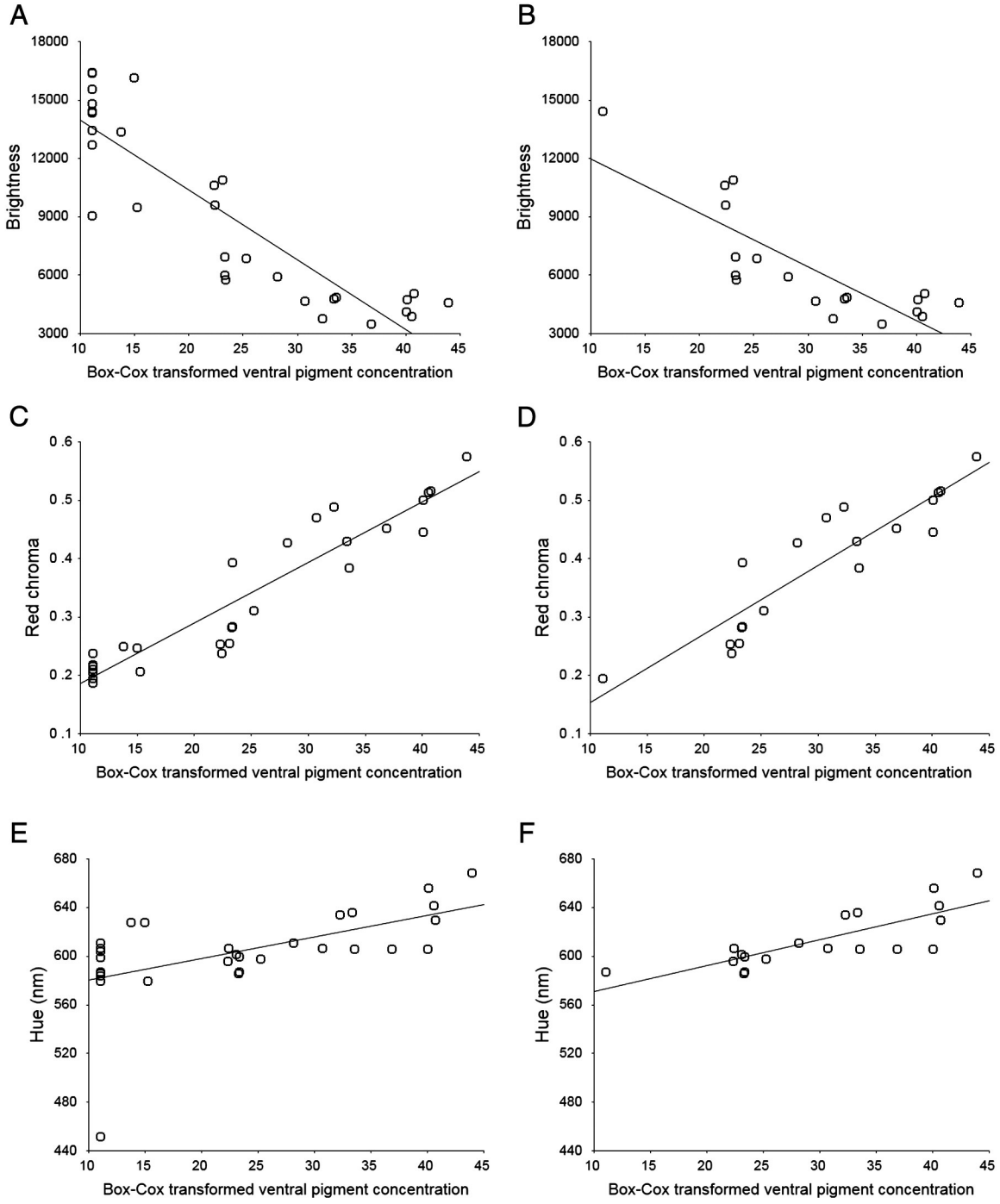

Fig. 2. Linear regressions between color parameters and pigment concentration in ventral tail skin of *Acanthodactylus erythrurus* lizards. (A) Brightness, (B) red chroma and (C) hue for all 30 lizards (brightness: $y = 17544 - 358\,x$, $r^2 = 0.787$; red chroma: $y = 0.0829 + 0.0104\,x$, $r^2 = 0.894$; hue: $y = 562.50 + 1.77\,x$, $r^2 = 0.304$; $F_{1,28} \geq 12.22$, $p \leq 0.0016$ in the three tests). (D) Brightness, (E) red chroma and (F) hue for juvenile lizards only (brightness: $y = 14724 - 276\,x$, $r^2 = 0.672$; red chroma: $y = 0.0352 + 0.0118\,x$, $r^2 = 0.831$; hue: $y = 549.81 + 2.13\,x$, $r^2 = 0.613$; $F_{1,17} \geq 26.96$, $p < 0.0001$ in the three tests). All six tests are statistically significant after sequential Bonferroni correction (see Statistical analyses for details). Pigment concentrations are relative values without units (see Pigment determination and quantification).

that the apparent red color was due to pterins, specifically drosopterin, as found by comparison with *Drosophila* flies.

Pigment concentration in ventral tail skin was significantly related to the three color parameters (Fig. 2). Specifically, higher pigment concentration resulted in a darker, more red-saturated, redder (less orange) color. Unexpectedly, pigment concentrations in ventral and dorsal tail skin were positively related (Table 1), although it was higher in ventral skin ($z = 2.38, n = 8, p = 0.017$). As a result, ventral coloration was significantly related to pigment concentration in dorsal tail skin (Table 1). Muscle pigment concentration was very low or completely absent, and unrelated to dorsal or ventral pigment concentrations or to ventral coloration (Table 1).

4. Discussion

In contrast to carotenoids, pterins are not obtained directly through the diet but synthesized from guanosine triphosphate within the cells via a cyclohydrolase enzyme reaction (Ziegler, 1965; Nichol et al., 1985). Very little is known about the physiological effects of pterins in vivo, but they seem to be involved in oxidative processes (McGraw, 2005), both as free-radical scavengers and free-radical promoters, depending on particular conditions (Oettl and Reibnegger, 2002). The net effect of these antagonistic functions on organisms has not been well studied. Some pterins, such as neopterin, are released from macrophages during activation of the immune system (Huber et al., 1984), possibly to protect immune cells from oxidative damage (Gieseg et al., 2001). Interestingly, a neopterin-like compound (dihydroneopterin triphosphate) is a precursor of drosopterin (Dorsett et al., 1979; Dorsett and Jacobson, 1982), the pterin responsible for orange/red coloration in *A. erythrurus*. The specific physiological functions of drosopterin have never been studied.

Orange/red coloration in *A. erythrurus* has different functions at different age classes (Fresnillo, 2014). In hatchlings, it is an antipredatory mechanism, diverting predator attacks from vital body parts to the autotomizable tail (Fresnillo et al., 2015b). In juveniles, it is an intraspecific communication signal which reduces aggression from conspecific adults (Fresnillo et al., 2015a). In adult females, it is associated with their reproductive cycle and probably functions as a sexual ornament (Belliure, Cuervo and Fresnillo, unpublished results). Using orange/red coloration to signal phenotypic condition would be useful to adult females looking for sexual partners, but its usefulness is not so obvious in juveniles, because *A. erythrurus* does not exhibit parental care. Possible competition among juveniles for social status or for resources has never been investigated in this species.

Ventral tail coloration in *A. erythrurus* reflected pterin concentration in ventral tail skin fairly closely, with more pigmented individuals showing darker, redder color. This suggests that pterins in chromatophores are arranged in such a way that they are visible, at least in the ventral part of the tail, with no or little interference from structural elements. These relationships, and the fact that carotenoids were not found in ventral tail skin, also suggest that pterins are the only pigments responsible for orange/red coloration in this species, which, in turn, might favor the visibility of the pterins. Studies of the relationship between coloration and pterin concentration in other vertebrate species with orange/red-colored skin have provided mixed results, possibly because all these other species also have carotenoids (Grether et al., 2005; Clotfelter et al., 2007; Steffen and McGraw, 2009; Weiss et al., 2012). In some species of lizards, the ratio between pterin concentrations in colored and non-colored skin areas is significantly related to coloration, although pterin concentration itself is only marginally positively related to coloration (Weiss et al., 2012). In other lizard species chroma, but not brightness, is significantly related to pterin concentration (Steffen and McGraw, 2009). In some species of fish, redness is negatively related to pterin concentration, not positively (Clotfelter et al., 2007), while in others the relationships are complex and heavily dependent on carotenoid concentration (Grether et al., 2005). Obviously, other factors such as integumentary nanostructure and/or other types of pigments might have contributed to modulate color expression (Steffen and McGraw, 2009; Weiss et al., 2012).

Pterin concentration was higher in the ventral than in the dorsal tail skin, but both concentrations were positively correlated, giving rise to an association between dorsal pterin concentration and ventral coloration. Dorsal tail color was not quantified in this study, but other studies in the same species suggest that ventral and dorsal tail colors (specifically red chroma) are positively correlated, at least in juveniles (Fresnillo, Belliure and Cuervo, unpublished results). One possible explanation for the presence of pterins in dorsal tail skin is that pterins accumulate in the skin of the whole tail, even if only needed in the ventral part. Some dorsal skin characteristics, such as its nanostructure, the distribution of pterins within chromatophores, the shape and size of chromatophores or the presence of other pigments (e.g. melanin), might cover the pterins, making dorsal parts less conspicuously colored. If this is the case, pterin concealment might be an adaptation reducing detectability by predators. Pigment accumulation seemed to be restricted to the skin, because tail muscular tissue lacked pterins or showed very low concentration. In any case, dorsal pterin concentration was calculated in a small number of individuals ($n = 8$), so results regarding the dorsal part of the tail should be considered with caution. Moreover, dorsal tail coloration was not quantified, which is a limitation of the study. Therefore, further studies with larger sample size and with spectrophotometric measurements of dorsal tail skin in individuals for which pigment concentration is determined are needed to confirm our preliminary findings.

In this study, we found that skin coloration reflected pigment concentration for a pterin-based orange/red- or white-colored body part in a lizard species. Moreover, according to our preliminary results on dorsal tail skin, we can speculate about a scenario in which the need to color a skin patch increases pigment concentration not only in that patch, but also in other body parts. However, the relationship between skin coloration and pigment concentration differs depending on the body part considered. In some parts (e.g. ventral tail), skin coloration accurately reflects pterin concentration and might have a signaling function, but in others (e.g. dorsal tail), despite also presenting pterins, skin coloration is modified by other skin characteristics, possibly as an anti-predatory mechanism. Future studies with appropriate sample size and methodology (measuring also dorsal tail coloration) will have to confirm this hypothetical scenario.

Table 1
Spearman correlations between ventral, dorsal, and muscle tail pigment concentrations and ventral tail brightness, red chroma and hue in a subsample of eight *Acanthodactylus erythrurus* lizards.

	Ventral pigment concentration	Dorsal pigment concentration	Muscle pigment concentration
Ventral brightness	$r = -0.905^{**}$	$r = -0.857^{**}$	$r = -0.265$
Ventral red chroma	$r = 0.929^{***}$	$r = 0.929^{***}$	$r = 0.234$
Ventral hue	$r = 0.886^{**}$	$r = 0.731^{*}$	$r = 0.267$
Muscle pigment concentration	$r = 0.405$	$r = 0.094$	
Dorsal pigment concentration	$r = 0.786^{*}$		

Correlations between pigment concentrations in different parts of the tail are also shown. All p-values < 0.05 are statistically significant after sequential Bonferroni correction except the relationship between ventral hue and dorsal pigment concentration (see Statistical analyses for details).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpb.2015.11.011.

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