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# Coloration reflects skin pterin concentration in a red-tailed lizard

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A R T I C L E   I N F O

A B S T R A C T

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 also been 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 accurately reflects pigment concentration or may not 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