Skin Pigmentation of Stellagama stellio (L. 1758) (Reptilia: Agamidae) Depends on Climate Conditions and Altitude

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Abstract: We studied skin pigmentation characteristics of the starred agama, Stellagama stellio, at different altitudes and climate conditions from Anatolia, Turkey. The epidermis was composed of stratum corneum and stratum basale, while the dermis occupied a much larger area. Melanophores were only found in the dermis and melanin pigments exhibited different distribution patterns in cells. Our results indicated that the skin pigmentation of S. stellio varied among populations depending on the altitude and climatic conditions. At higher altitudes, the melanophores in dorsal skin were larger and its melanin pigments aggregated in melanophores. The aggregation of the melanin pigments indicated the skin had lighter colour. The melanin pigments tended to be dispersed at lower altitudes. The skin of populations inhabiting hot climates and lower altitudes was more pigmented, while populations from steppe and higher altitudes exhibit lower pigmentation when comparing melanophores areas.

Key words: Starred agama, skin histology, pigmentation, altitude, climate, Anatolia

Introduction

The activity patterns of animals depend on both biotic (e.g. ecological interrelationships, vegetation structure) and abiotic (e.g. altitude, environmental temperature, moisture) factors in the environment they inhabit (Zamora-Camacho et al. 2013). Reptiles, as ectotherms, are strongly dependent on environmental temperature for maintaining their physiology (Bennet 1980). The variation of temperature along with the altitudinal gradients could be the cause of the differences in lizards’ thermoregulatory needs, behaviour and phenology (ADOLPH & PORTER 1993, CHETTRI et al. 2010, GUTIÉRREZ et al. 2010).

Generally, skin consists of three main layers: epidermis, dermis and hypodermis. The epidermis is composed of keratinised stratified squamous epithelium (ROSS & PAWLINA 2011). The outer keratinised surface of the epidermis consists of dead cells produced by the cells of the deepest epidermal layer during the maturation (IRISH et al. 1988). The non-mammalian dermis may contain connective tissue, cells, blood vessels and related ducts. Paraphyletic reptiles, as a group, have been characterised as devoid of glands on the dermis, in contrast with the amphibians (Romer 1949, Hiller & Werner 2008).

Reptiles’ integument has adapted to arid conditions through some alterations such as increasing of keratinisation, pigmentation and of dorsal-ventral skin thickness (Darwish 2012). Therefore, the skin of most reptiles including chelonians, crocodiles and lizards is hard and contains scales (Toni et al. 2007). Histological organisation of the integument can vary among species (AliBardi 2003, CHANG et al. 2009, Darwish 2012).

Chromatophores are pigment-containing and light-reflecting cells or groups of specialised skin cells in ectothermic vertebrates (Oliveira & Franco-Bellusci 2012). Melanophores are a kind of chromatophores, which produce and contain
black and brown melanin pigments and have some dendrite-like extensions (Kierszenbaum 2002, Oliveira & Franco-Bellusc 2012). These cells are located in stratum basale of the epidermis in endotherms, whereas in ectotherms they are located mostly in the deepest layer of dermis. Melanin protects the organism against the damaging effects of non-ionizing ultraviolet irradiation (Ross & Pawlina 2011, Cichorek et al. 2013).

Reptile pigmentation originates from physical and cellular processes (Cooper & Greenberg 1992). Colour patterns in reptiles may be affected by reproductive tactics, hormones, physiological stress and ageing (Ollson et al. 2013). The structural (physical) colouration derives from the interaction of light with ordered collagen fibrils or with the reflecting platelets of chromatophores present in the dermis. Other types of skin colouration are an adaptive response to the surrounding environment and derive from rapid or slow (physiology-based) re-distribution of melanosomes within dural melanophores or from the long (morphology-based) accumulation of melanosomes in the epidermis from epidermal melanocytes (Alibardi 2011).

The starred agama, Stellagama stellio (Linnaeus, 1758), is widely distributed along the Mediterranean islands of Turkey and Greece and also in Anatolia, Syria, Eastern Iraq, Lebanon, Israel and Jordan to Lower Egypt and Northern Saudi Arabia (Daan 1967, Başoğlu & Baran 1977, Almog et al. 2005). Agamas generally prefer rocky areas with limited vegetation and are well adapted to semi-desert and desert areas (Başoğlu & Baran 1977). In this study, we investigated skin structure and pigmentation variations of S. stellio among populations found at different altitudes and climate conditions in Anatolia, Turkey.

Materials and Methods

Study area: Ten individuals (five males and five females) were collected in April–May 2010 from each of four localities: Hatay (Samandağ, 1 m a.s.l.), Denizli (Hierapolis Ancient City Pamukkale, 363 m a.s.l.), Konya (1005 m a.s.l.) and Isparta (Bağkonak Village, Yalvaç, 1345 m a.s.l.). The individuals had been previously collected for a study on their bone histology and age structure (Kumaş & Ayaz 2014).

Climate conditions experienced by the population were classified according to the Köppen-Geiger climate classification (WMKGCC 2016). Hatay and Denizli have warm temperate climate with hot summer (CSa). Isparta has warm temperate climate with warm summer (CSb). Konya’s climate is classified as cold steppe climate (BSk). Climate data related to mean temperature and rainfall (1987–2011) of the study areas were obtained from the Turkish State Meteorological Service (Table 1).

Histological Analysis: As a part of previous studies, all individuals were already fixed with 96% ethanol. Small parts of skin tissue were dissected from both dorsal and ventral regions and were prepared for histological analysis. Five-µm-thick longitudinal sections were stained with Harris Haematoxylin & Eosin and Mallory’s trichrome and examined under a light microscope. Melanophore areas were measured using a Nikon Eclipse i5 light microscope with a Nikon DS-Fi1c camera and Nikon NIS Elements version 4.0 image analysis systems (Nikon Instruments Inc., Tokyo, Japan). All data were compared statistically using ANOVA (One-way analysis of variance: Tukey test). The alpha was set at 0.05.

Results

General skin structure: Each of the three skin layers, epidermis, dermis and hypodermis, had different tissue characteristics. Epidermis, the uppermost layer of the skin, contained two layers, called stratum corneum and stratum germinativum (stratum basale). Two distinct keratin layers were observed in the stratum corneum: an uppermost light-coloured layer...
and a darker-coloured one. We observed an artificial gap between these layers resulting from the preparation (Fig. 1). The cellular portion of the epidermis consisted of a single layer of stratum germinativum (stratum basale) layer, composed of simple columnar or high cuboidal cells, called keratinocytes, located on the basal lamina. Epidermal melanophores were not observed between epithelial cells. The epidermis was substantially thinner than the dermis.

The connective tissue in the dermis did not project into the epidermis, so we did not observe dermal papillae. In the dermis, there were two different layers, including papillary dermis and reticular dermis, according to the distribution of collagen. Although no dermal papillary was observed in the dermis, collagen fibres close to the epidermis, which were called papillary dermis, showed a different appearance compared to those of the reticular dermis located in the deeper part of the dermis. The papillary dermis contained thin and randomly distributed loose connective tissue, whereas the reticular dermis was composed of dense connective tissue (Fig. 2). Blood vessels were seen in papillary dermis (Fig. 3). As expected, epidermal skin appendages, including secretion channels and sebaceous glands, were not found within the dermis. The melanophores were only found in the dermis layer and not found in the epidermis. The large melanophores were extended from the reticular layer to the papillary dermis and reached stratum basale (Fig. 4a).

In Konya individuals, the pigment granules were rather concentrated in melanophores and the cells found between the stratum basale and papillary dermis layers. In a single female, we observed that melanophores reached from the papillary dermis layer to stratum basale with pseudopodia. Medium-sized melanophores (11.52 ± 2.14 µm²) were observed in three females and one male between the papillary dermis and the reticular dermis, whereas a small number of black and large melanophores (38.33 ± 9.84 µm²) were observed in the reticular dermis (Fig. 4b).

The melanin pigments were parallel to the epithelial surface in individuals that were collected from Isparta. They formed a thick and dense layer in the papillary dermis (144.01 ± 12.36 µm², Fig. 4c). In two females, some of the melanophores reached stratum basale by pseudopodia. In some individuals, there were medium-sized melanophores between the reticular and the papillary dermis (10.98 ± 2.14 µm²). Melanophores were mostly observed in the reticular dermis, whereas the ones that had dispersing

**Ventral Skin Histology:** The histological structures of the epidermis and hypodermis layers were found to have similar features. However, there were some differences among populations in terms of melanocyte size and pigment distribution.

The melanophores of the individuals from the Denizli region, located between stratum basale of the epidermis and the papillary dermis had dispersed pigments. Those were seen as a thin layer (Mean area ± SD = 5.84 ± 0.86 µm²). Large melanophores (639.19 ± 23.45 µm²) extended from the reticular layer to the papillary dermis with pseudopodia and then reached stratum basale (Fig. 4a).

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**Fig. 1.** Histological view of the ventral skin of a male *Stellagama stellio* from Isparta. Blue arrows indicate bundles of collagen fibres. Stain: Harris Haematoxylin-Eosin. Scale-bar: 31.94 µm

**Fig. 2.** Histological view of the dorsal skin of a female *Stellagama stellio* from Denizli. Stain: Harris Haematoxylin-Eosin. Scale-bar: 31.94 µm
pigments were mostly located between the papillary dermis and stratum basale. Melanophores formed a very thin layer in the papillary dermis (38.93 ± 4.83 µm², Fig. 4d) in individuals that were collected from Hatay. Only two males had, respectively, one and two medium-sized black melanophores in the reticular dermis (43.56 ± 5.78 µm², Fig. 3).

**Dorsal Skin Histology:** The dispersing melanin pigments were not found under stratum basale in individuals that were collected from Hatay. Only two males had, respectively, one and two medium-sized black melanophores in the reticular dermis (43.56 ± 5.78 µm², Fig. 3).

**Melanophores were larger than those of other populations (50.72 ± 17.76 µm² and 543.12 ± 39.45 µm², respectively) and those cells extended from the papillary dermis to stratum basale by a large number of pseudopodia. In the papillary dermis densely aggregated pigments in melanophores were observed (Fig. 5b). The melanophores in the reticular dermis (543.12 ± 47.26 µm²) were larger than those in the papillary dermis (4.09 ± 1.06 µm²) in individuals collected from Isparta (Fig. 5c).**

In Hatay individuals, medium-sized melanophores (14.09 ± 2.38 µm²) were mostly located in the papillary dermis and they reached stratum basale by their pseudopodia. Melanophores, which also appeared in the reticular dermis (8.64 ± 1.14 µm²), were smaller than those of the papillary dermis (Fig. 5d).

In populations living under WMKGCC categories “Warm temperate climates with warm summer” and “Local steppe climate” conditions, pigmentation of skin was higher than that of populations living under “Warm temperate climates with hot summer” (F = 45.521; df = 97, P < 0.0001). The largest mean area of melanophores located in the reticular dermis was obtained from Denizli and Isparta populations that live in warm temperate climatic condition.

At higher altitudes (Konya and Isparta), the melanophores in dorsal skin were larger and melanin pigments were aggregated in the cells. The aggregation of the melanin pigments indicated that the skin had a light colour. The melanin pigments tended to disperse at lower altitudes (Hatay and

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**Fig. 3.** Longitudinal section of the ventral skin in a male *Stellagama stellio* from Hatay. Blue arrows indicate the melanophores. BV: blood vessels. Stain: Harris Haematoxylin-Eosin, Scale-bar: 7.78 µm

**Fig. 4.** Longitudinal sections of the ventral skin of *Stellagama stellio* from [a] Denizli. Stain: Mallory’s trichrome, [b] Konya, [c] Isparta and [d] Hatay. Blue arrows indicate the melanophores; the red circle indicates dispersion of melanin in the melanophores. Stain: Harris Haematoxylin-Eosin. Scale-bar: 7.78 µm

**Fig. 5.** Longitudinal sections of the dorsal skin of *Stellagama stellio* from [a] Denizli, [b] Konya, [c] Isparta, and [d] Hatay (M: melanophores, Nc: Nucleus). Stain: Harris Haematoxylin-Eosin. Scale-bar: 7.78 µm
Denizli) which meant that the dorsal skin was darker at lower altitudes. The skin of populations inhabiting hot climates and lower altitudes (Hatay and Denizli) was more pigmented than the one of other populations. Even though the ventral skin generally exhibited similar structure with the dorsal skin, in Isparta population the melanophores of the ventral skin were close to the epidermis unlike dorsal skin. This could be caused by differences in average temperature. According to a melanophore area comparison, the skin of individuals living in hot areas and at low altitudes was more pigmented, while the steppe and higher altitudes populations exhibited lower pigmentation.

**Discussion**

Studying different altitude gradients is an opportunity to understand adaptations to variable environmental factors. The variables with the highest effect on an organism regarding altitude are temperature and ultraviolet radiation which respectively decrease and increase with altitude (Reguera et al. 2014). Therefore, reptiles distributed in lowlands and highlands are expected to have different phenotype due to the different climatic conditions they experience (Castilla et al. 1999).

Melanophores are light-absorbing pigment cells that produce black or brown colours, found both in the epidermis and the dermis. The spatial arrangement and architectural combination of these pigment cells can produce different skin colours in reptiles (Morrison 1995, Morrison et al. 1996). The presence of epidermal melanophores may vary with age, sex, skin surface location and geographical distribution (Cooper & Greenberg 1992). The presence of epidermal melanophores and dermal chromatophores was shown for Pléstiodon laticutatus (see Kuriyama et al. 2006) and Sphenodon punctatus (see Alibardi 2011).

Epidermal melanophores allow transfer of melanosomes into the keratinocytes (Alibardi 2011). Melanophores of poikilotherms cause dark backgrounds and generally contain dispersed melanosomes. When melanin pigments aggregate in the perinuclear region they cause light colour (Cooper & Greenberg 1992). Desert lizards have typically light colour at high temperatures, becoming darker only at very low body temperatures (Atsatt 1939).

We found that *Stellagama stellio* had dorsal and ventral skin pigmentation variations along the altitudinal gradient. We detected that melanin pigments aggregated within the melanophores in the populations living in warm temperature climate with hot summers, whereas degraded within the melanophores in the populations living in warm temperature climate with warm summers and in local steppe climate. To date, four hypotheses have been proposed to explain the occurrence of dorsal and ventral colour polymorphism: thermoregulatory advantages in different microclimatic conditions (Sherbrook et al. 1994, Lepetz et al. 2009, Sacchi et al. 2012), mimesis when different morphs are advantageous at different habitats (Stuart-Fox et al. 2004, Roseblum 2006, Capula et al. 2009), protection from ultraviolet radiation (Clussella-Trullas et al. 2008, Reguera et al. 2014) and sexual selection (Stuart-Fox & Ord 2004). Among these hypotheses, we only tested whether altitude dependent temperature variables elicit colour change.

Our results showed that darker dorsal and ventral colouration at higher altitudes did not support the “thermal melanism hypothesis”, which predicts that dark individuals are advantageous in cool climates as they heat faster and reach higher equilibrium temperatures than lighter individuals (Clussella-Trullas et al. 2007, 2008). Gvoždík (1999) compared heating rate, body size and body condition of Zootoca vivipara and found that the species did not confirm thermoregulatory advantage hypothesis. Heating up faster is important for ectotherms especially for reptiles because in this case thermoregulation is connected to solar radiation (Vitt & Caldwell 2009). Our results supported the “protection against ultraviolet damage hypothesis” (Porter & Norris 1969), which helps us to explain why *S. stellio* was darker at increased altitudes, based on increasing UV radiation intensity with elevation (Porter & Norris 1969). Solar radiation is necessary for thermoregulation but it can be harmful at high altitudes (Sola et al. 2008) because the thinner atmosphere allows more UV radiation to reach the ground (Reguera et al. 2014). High levels of UV radiation damages DNA (Ravant et al. 2001), eggs and embryos (Marquis et al. 2008) and can cause cellular oxidative stress (Chang & Zheng 2003).

The other two hypotheses about dorsal and ventral colour variation depending on altitude, the “cryptic-colouration hypothesis” (Reguera et al. 2014) and the “sexual selection” (Stuart-Fox & Ord 2004) were not examined in our study. Further analysis would suggest the most effective mechanism for altitude-dependent polymorphic colouration of *S. stellio*.

There are many studies on dorsal and ventral colour polymorphism, not only in terms of abiotic factors and environmental changes but also with regard to genetic factors, such as phenotypic plastic-
ity (ROSEMBLUM 2005) and melanocortin-1 receptor (e.g., MARKKLUND et al. 1996, DECREST et al. 2008, HUANG et al. 2014, FULGIONE et al. 2015). Melanin pigments provide the most widespread source of colouration in vertebrates. The melanin-based colouration could be the result of both mutations in the melanocortin-1 receptor gene and of differential expression of the same gene for a wide range of vertebrates (e.g., SKOGLUND & HÖGLUND 2010, VÅGE et al. 2014, ZHANG et al. 2014, FULGIONE et al. 2015). However, this mechanism is still poorly known in lizards and some populations can exhibit a high variation of melanism (FULGIONE et al. 2015). Future melanin-based studies of agamid lizards could help in understanding the skin colouration mechanisms and the environmental factors that effect skin colour polymorphism.

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