PŘÍLOHY

Publikace zahrnuté do dizertační práce

I. **Abramjan A, Bauerová A, Somerová B & Frynta D. 2015.** Why is the tongue of blue-tongued skinks blue? Reflectance of lingual surface and its consequences for visual perception by conspecifics and predators. *Science of Nature 102:42*

II. **Abramjan A, Baranová V, Frýdlová P, Landová E & Frynta D.** Ultraviolet reflectance and pattern properties in Leopard geckos (*Eublepharis macularius*). *Submitted to Behavioral Ecology and Sociobiology*

III. **Abramjan A, Žampachová B, Rádlová S, Landová E & Frynta D.** Snakes, ‘flags’ and contrasts: analysing conspicuousness of aposematic pattern through eye-tracking and visual modelling. *Submitted to Biological Journal of the Linnean Society*

IV. **Abramjan A, Frýdlová P, Jančúchová-Lásková J, Suchomelová P, Landová E, Yavruyan E & Frynta D. 2019.** Comparing developmental stability in unisexual and bisexual rock lizards of the genus *Darevskia*. *Evolution & Development: 1*‑*13.*

Připravovaný rukopis (*manuscript in prep.*)

V. **Abramjan A, Frynta D.** Are parthenogenetic females less colourful than sexual ones? Evaluating UV-blue traits in the lizards of the genus *Darevskia.*

Prohlášení spoluautorů:

Jménem svým i ostatních spoluautorů potvrzuji, že autorský podíl Andrana Abramjana na publikacích a manuskriptech, které jsou součástí jeho disertační práce, odpovídá počtu spoluautorů a jejich pořadí. Potvrzuji též, že se Andran Abramjan podílel na všech fázích přípravy těchto publikací, včetně sběru a zpracování dat, statistického zhodnocení, formulaci výsledků, sepisování vlastního textu rukopisů a nakonec i finálních úpravách v průběhu recenzního a publikačního procesu.

V Praze dne 24.6.2019

Daniel Frynta

I

Why is the tongue of blue-tongued skinks blue? Reflectance of lingual surface and its consequences for visual perception by conspecifics and predators

Andran Abramjan, Anna Bauerová, Barbora Somerová, Daniel Frynta (2015)

Science of Nature (Naturwissenschaften) 102:42

Sci Nat (2015) 102:42
DOI 10.1007/s00114-015-1293-4

ORIGINAL PAPER

Why is the tongue of blue-tongued skinks blue? Reflectance of lingual surface and its consequences for visual perception by conspecifics and predators

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Received: 24 March 2015 / Revised: 30 June 2015 / Accepted: 3 July 2015

Abstract Blue-tongued skinks of the genus *Tiliqua* (Scincidae) are characterized by their large blue melanin-pigmented tongues, often displayed during open-mouth threats, when the animal feels endangered. It is not clear whether this unusual coloration is a direct anti-predation adaptation or it may rather serve intraspecific communication, as ultraviolet-blue color is a frequent visual signal in a number of lizard species. We used spectrophotometry and visual modeling to compare blue tongues of *Tiliqua gigas* with tongues and skin coloration of other lizard species, and to examine their appearance through the eyes of both the conspecifics and avian predators. Our results show that (1) the tongue coloration is probably not substantially influenced by the amount of melanin in the skin, (2) lingual and oral tissues are UV-reflective in general, with blue colored tongues having chromatic qualities similar to UV-blue skin patches of other lizard species, (3) UV-blue tongues are more conspicuous than pink tongues, especially in the visual model of conspecifics. We hypothesize that blue tongues may possibly serve as a semantic (honest) signal analogous to UV-blue skin patches of other lizard species due to greater UV-bias in the vision of diurnal lizards. Regarding the social behavior and high aggressiveness in *Tiliqua* and their relatives, such signal might serve, e.g., in intraspecific long-distance communication between conspecifics in order to avoid aggression, and its anti-predation effect may only be a secondary function (exaptation).

Keywords Coloration · Signaling · Lizards · Evolution · *Tiliqua*

Communicated by: Sven Thatje

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Introduction

Color signals play an important role in both intra- and interspecific communication in a number of animal species. Apart from serving as cues for individual or species recognition, they cover a variety of semantic meanings (Williams and Rand 1977; Losos 1985; Couldridge and Alexander 2002; Creel and Creel 2002). The size or intensity of particular coloration can correlate with the hierarchic status of its bearer and/or indicate his health or aggressiveness. This is common in males of many fish, bird, or reptile species which indicate in this way their fighting ability to their competitors. This influences their mating success and therefore, the corresponding traits are often subject to sexual selection (Fernald and Hirata 1977; Andersson et al. 1998; Sinervo et al. 2000; Senar 2006; Hamilton et al. 2013). Conspicuous colors and patterns can also serve to deter potential predators. Aposematic patterns are usually present on the animal's body surface, being exposed either permanently (e.g., in coral snakes), or only at the moment of threat, which is typical for otherwise cryptic species (e.g., in fire-bellied toads). Some species rely on a strategy of startling the predator for a moment, flashing suddenly a hidden warning sign and thus gaining some time to escape (e.g., sunbitterns, frilled lizards or some species of butterflies; Ruxton et al. 2004; Hill and McGraw 2006; Hamilton et al. 2013).

Within the wide variety of color signals, blue coloration deserves special attention, as it is relatively uncommon among vertebrates, but mostly has a semantic function (Bagnara et al. 2007; Perez i de Lanuza and Font 2010; Umbers 2013). In this case, vision and coloration are related in a sense that many animals having blue patches on their surfaces are also capable of seeing ultraviolet light. Patches that we perceive as blue are in fact often UV-blue, as their peak reflectance is mostly in the UV range. Such signs are therefore conspicuous to these species, allowing them to communicate through this channel without being detected by UV insensitive predators (Endler 1992; Håstad et al. 2005; Lind et al. 2013; Marshall and Stevens 2014). UV-blue crowns in blue tits or throats, lateral eyespots, and outer ventral scales in many lacertids (e.g., *Timon*, *Gallotia*, *Lacerta*, *Podarcis* etc.) represent not only intraspecific recognition traits (they can be sexually dichromatic in the UV spectrum in many species), but also serve as honest signals of male quality (Andersson et al. 1998; Font et al. 2009; Bajer et al. 2011; Molina-Borja et al. 2006). In African flat lizards of the genus *Platysaurus* (Cordylidae) as well as in the lacertids, the intensity of UV-blue coloration positively correlates with the aggressiveness and the ability of males to mate successfully (Whiting et al. 2006; Font et al. 2009; Fleishman et al. 2011; Perez i de Lanuza et al. Page: 5 2014a). On the other hand, a blue color is very rarely involved in warning signals and its potential aposematic function remains questionable (Bagnara et al. 2007; Umbers 2013).

Unlike most lizards, blue-tongued skinks of the genus *Tiliqua* (Gray 1825) are special by their non-dermal *warning* blue sign. Current taxonomy recognizes seven species of these mostly robust diurnal omnivorous skinks (*Tiliqua scincoides*, *Tiliqua gigas*, *Tiliqua multifasciata*, *Tiliqua nigrolutea*, *Tiliqua occipitalis*, *Tiliqua rugosa*, *Tiliqua adelaidensis*) distributed throughout Australia, eastern Indonesia, and Papua New Guinea (Shea 2006; Gardner et al. 2008; Cogger 2014). As their name suggests, they are characterized by their large blue fleshy tongues, which are often exposed when the animal feels endangered or irritated. The skink widely opens its mouth and startles a rival or predator by exposing its tongue either inside the mouth by raising its base while keeping the tip down or by sticking the entire tongue out. This can be accompanied by body inflating, hissing, or lunging against the enemy (Carpenter and Murphy 1978; Murray and Bull 2004; Brown 2012; Cogger 2014). *Tiliqua* skinks are frequently hunted by the birds of prey (Aumann 2001; McDonald et al. 2003; Olsen et al. 2010); other avian predators may include kookaburras or corvids, while reptilian predators are mostly elapid snakes and monitor lizards (Hauschild et al. 2000; Koenig et al. 2002; Fitzsimons 2011).

Regardless of lingual coloration, an open-mouth threat is a common reaction among animals (Vitt and Lacher 1981; Sherbrooke 1991; Langkilde and Shine 2005; Godfrey et al. 2012). Herrel (2000) notes that although the tongue's defensive use is quite obvious, it does not really explain the function or presence of the blue coloration. Bright blue tongues are found, e.g., in *T. scincoides* or *T. gigas*, while *T. rugosa* has almost a black tongue and juveniles of a closely related skink *Cyclodomorphus gerrardii* have blue tongues, whereas adults have them pink. Thus, the situation is more complicated than it may seem. Herrel (2000) proposes also alternative hypotheses that the blue coloration can be more crucial as a warning sign in young animals, or it can represent an honest signal in competing males during the mating season.

The latter hypothesis may be plausible as aggressiveness is common among individuals of the genus *Tiliqua* and they are capable of wounding each other fatally (Graves and Halpern 1991; Shea et al. 2005; Godfrey et al. 2012). Males of *T. scincoides* occupy core areas which they defend against other males, while females tolerate each other and their home ranges considerably overlap (Price-Rees et al. 2012). Similar social patterns were also observed in *T. rugosa*, where solitary males were more aggressive than males having bonds with females (Kerr and Bull 2006). For these reasons one would naturally expect the need for advanced communication between blue-tongued skinks in order to avoid aggression. Abbate et al. (2009) have confirmed a presence of melanin in the tongue of *T. scincoides*. Since melanin pigmentation is sometimes positively correlated with dominance or aggressiveness in various species of birds or reptiles (McGraw et al. 2003; Jawor and Breitwisch 2003; Senar 2006; Mafli et al. 2011; Plasman et al. 2015), the *honest signal* hypothesis does not seem unlikely in this case, as the tongue tinges also vary among individuals of the same species. *T. scincoides* or *T. gigas* may have pale gray, cobalt blue or dark (nearly black) blue tongues, so their tongues' intensity or darkness may reflect their status or health.

In fact, pigmented tongues can be found in most species closely related to the genus *Tiliqua* with the exception of *T. adelaidensis* and *Corucia zebrata*, the former being a considerably derived species and the latter being basal to the whole group consisting of the remaining genera: (*Egernia* (*Liopholis* (*Cyclodomorphus*, *Tiliqua*))) (Hutchinson et al. 1994; Shea 1995; Shea and Miller 1995; Pyron et al. 2013). Skinks of the genus *Cyclodomorphus* may have not only their tongues pigmented, but also their oral mucosa, reaching blue, blue-gray, or blue-black tinges (Shea 1995; Shea and Miller 1995). *Egernia* skinks have pigmented tongues as well, though not to such an extent or intensity, having for instance dark tips or an overall pale bluish tint. Variously tinged lingual and/or oral mucosa have also been observed in unrelated species of scincid, agamid, iguanid, gekkonid and other lizards (*Gerrhosaurus flavigularis*, *Gonocephalus chamaeleontinus*, *Amphibolurus muricatus*, *Rankinia diemensis*, *Chamaeleolis* sp., *Strophurus* sp. etc.) which had blue, black, yellow, or orange tongues or mouth interiors (Melville et al. 2004; Ayala-Varela and Omar 2010; Holáňová et al. 2012). The reason for such unusual coloration of lizard tongue is yet obscure.

The aim of our study was to explore qualities of the blue lingual coloration from the perspective of both the honest signal and the specific communication channel. Is the tongue color related to the overall body pigmentation, or is it an independent trait? Is there a *hidden* UV signal like in other lizards with blue coloration? Which recipient (reptile/bird, predator/conspecific) is this visual signal best adjusted to? To shed more light on these questions, we examined (1) possible correlations between tongue coloration and somatic traits (body length, melanin skin pigmentation), (2) the conspicuousness of blue tongues as it is perceived by two groups of animals with different visual systems: avian predators (mostly raptors; violet-sensitive vision) and conspecifics (diurnal lizards; UV-sensitive vision), and (3) color similarities between blue tongues of *Tiliqua* and (un) pigmented tongues and semantic UV-blue skin patches of other lizard species. For this purpose, we carried out spectrophotometric measurements of lingual, oral, and dermal surfaces and analyzed them using visual modeling.

Materials and methods

Spectrophotometric measurements

We measured 14 captive adult individuals of *T. gigas* imported legally into Europe from Indonesia. The stocks originated from surroundings of Merauke town, Irian Jaya province, Indonesia (SW of New Guinea Island). We assumed that in case of an adaptive function, the blue color of the tongue would be more advantageous than a pink one. Therefore, besides the blue tongue, we also measured the pink color of oral mucosa as an approximation of a pink tongue. We decided to take the skinks' back coloration as an example of background, against which the tongue color contrasts were to be calculated. As their dorsal side is considerably cryptic and no reliable and representative sample of substrates from the skinks' natural habitats was available, we chose their skin as a proxy of their natural environment color. It may reflect the palette of colors typical for their habitats, ranging from forest floors to dry semi-deserts (fallen wood, bark and leaves, dry vegetation, sandy soil, etc.) (Price-Rees et al. 2013; Cogger 2014). From the observed variation of the skinks' backs, four notably distinct shades were chosen as substitutes for their natural background colors: light brown (stripes), dark brown (stripes), orange-reddish (spots/stripes), and grayish (side of the head and neck) (Fig. 1).

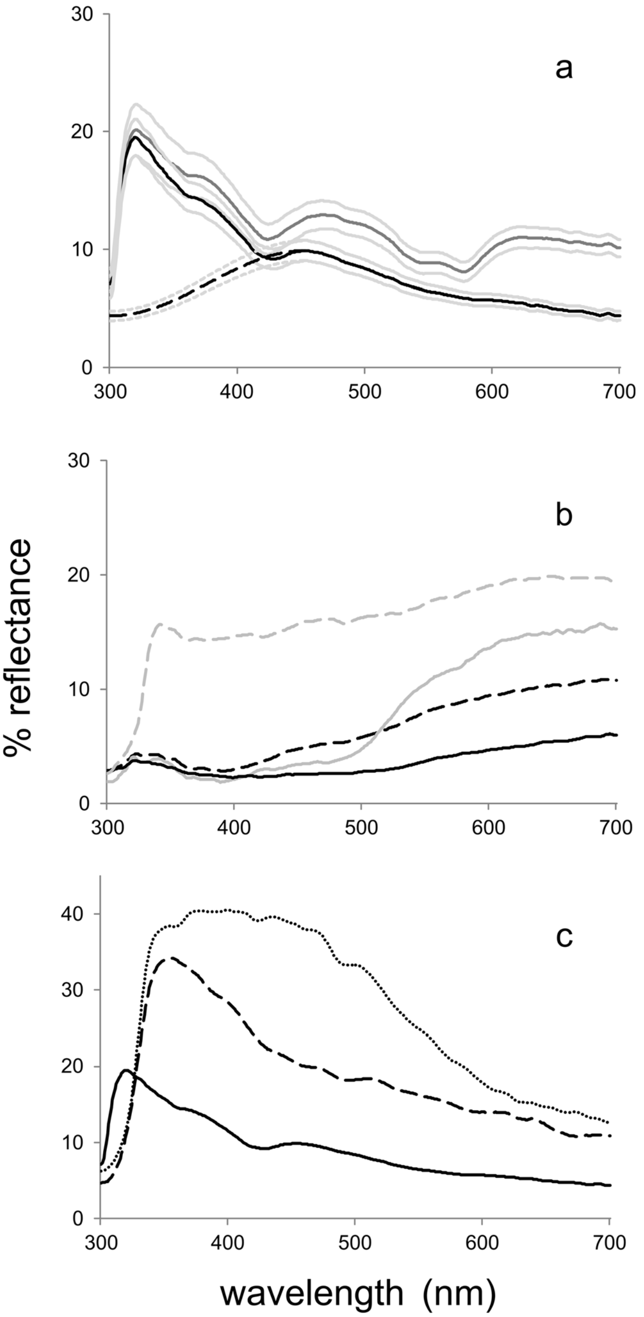

Fig. 1 Reflectance of lingual, oral and skin surfaces. **a** *T. gigas* (*n*=14). Mean reflectance of blue tongues (*solid black line*), mean reflectance of pink oral mucosa (*solid dark gray line*), manipulated part of spectra in the UV-negative set (*dashed black line*), ± SE (*light gray lines*). **b** Reflectance of background colors taken from skin samples of *T. gigas*: dark brown (*solid black line*), light brown (*dashed black line*), orange-reddish (*solid gray line*), grayish (dashed gray line). **c** Mean reflectance of blue tongues of *T. gigas* (*solid black line*), mean reflectance of blue shoulder spot of a single captive *G. galloti galloti* (*dashed black line*), mean reflectance of blue outer ventral scales (OVS) of a single *D. caucasica* (*dotted line*)

Color reflectance between 300 and 700 nm was measured with an OceanOptics USB4000 spectrophotometer and an UV-VIS Pulsed Xenon lamp source PX-2. The spectra were taken in a shaded room with a probe held in a constant 5 mm distance under a 45° angle to avoid possible specular effects caused by the glossy scales or moist surface of the tongue. The device was re-calibrated after every third measurement against an Ocean Optics WS-1 white standard and the probe was always sterilized with ethanol before measuring a new individual. Each color patch was measured three times and its mean reflectance was calculated. Skinks mostly opened their mouths spontaneously in a response to the irritation caused by our manipulation. Most of them kept their mouths open long enough for us to take measurements of the tongue and pink interior. Only when the skinks did not open their mouth themselves, we gently opened it. This seemed to be a sufficient impulse for the individual to keep its mouth open for a while.

As a comparison, we also measured tongues of *Egernia frerei*, *C. gerrardii* and *C. zebrata* (which are closely related to the genus *Tiliqua*; Pyron et al. 2013) and of an unrelated African skink *Trachylepis perrotetii*. With the exception of *C. zebrata*, whose tongue was wholly pink, all of them had their tongues at least partially pigmented: a dark gray tongue in *E. frerei*, a dark gray tip of the tongue in *T. perrotetii*, and an overall faintly bluish tint in *C. gerrardii* (Fig. 2). Each of these species was represented by a single individual.

To compare the blue tongue to UV-blue semantic coloration of other lizard species, we also acquired reflectance spectra from a captive *Gallotia galloti galloti* (shoulder spots) and *Darevskia caucasica* (outer ventral scales—OVS); single specimens in both cases (Fig. 1). Mean reflectance for other species was obtained from literature: *G. galloti galloti* (shoulder spots, *n*=26, Molina-Borja et al. 2006), *Gallotia galloti eisentrauti* (shoulder spots, *n*=34, Molina-Borja et al. 2006), *Timon lepidus* (lateral eyespot, *n*=14, Font et al. 2009), *Podarcis pityusensis* (OVS, *n*=40, Perez i de Lanuza and Font 2010), *Podarcis lilfordii kuligae* (both OVS and ventrum, *n*=47, Perez i de Lanuza and Font 2010) and *Platysaurus broadleyi* (throat, *n*=1, Whiting et al. 2006). The data were extracted with the WebPlotDigitizer (available on arohatgi.info/WebPlotDigitizer/app/ in December 2014). Curves for conspecific males and females were averaged into a single one to approach a *general* signal of the species. Unfortunately, morphology-based sexing of *Tiliqua* is known to be very unreliable (Brown 2012), and it was impossible to determine sex in most cases even with the ultrasound screening. Therefore, we could not look for the hypothetical sexual dichromatism in the coloration of their tongues.

Color processing

To compare whether the overall skink's body pigmentation correlates with its tongue color, we carried out the following procedure. We scanned each individual from its ventral and dorsal sides with a CanoScan 4400 F scanner at 600 dpi resolution, together with BST1 Color and Gray Control Chart as a reference of standardized colors. Both the skink's back and belly (including the top of the head and throat, respectively) were outlined and cut out from the scans in Photoshop CS6 (Fig. 2).

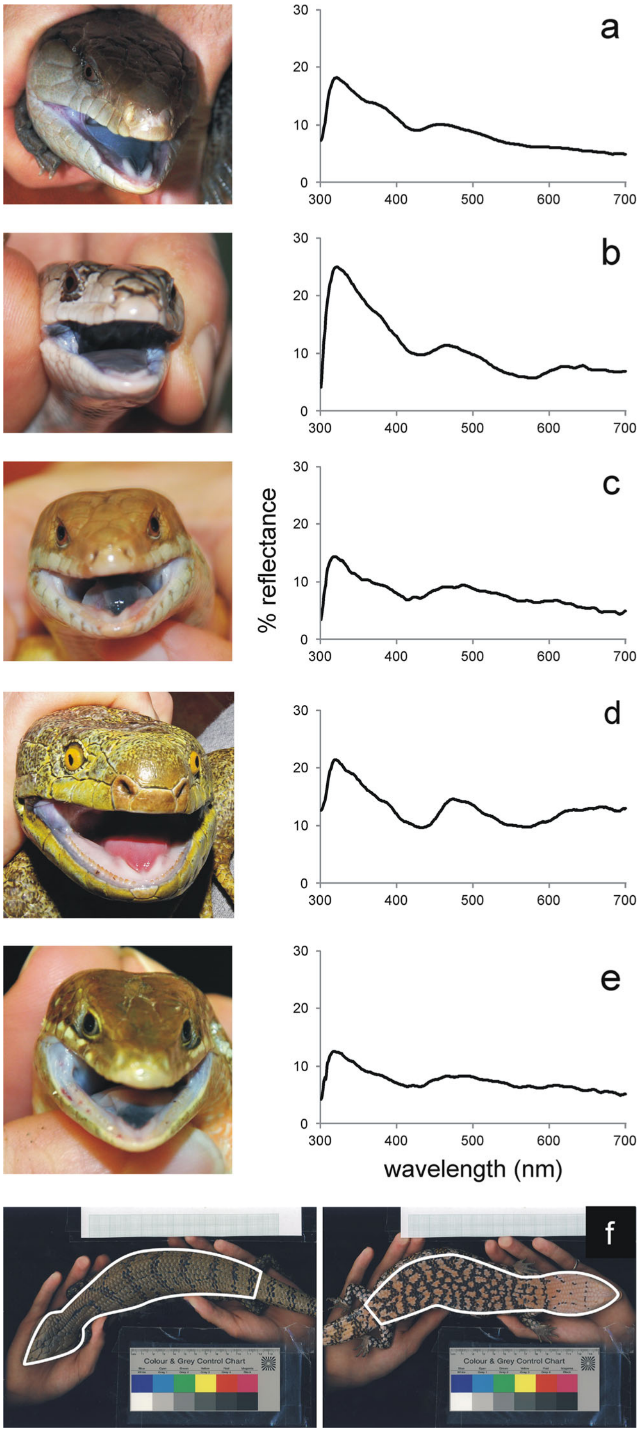

Fig. 2 Tongues and their reflectance spectra of species used in this study: **a** *T. gigas*, **b** *C. gerrardii*, **c** *E. frerei*, **d** *C. zebrata*, **e** *T. perrotetii*, **f** scan examples of dorsal and ventral sides of skinks; *white outline* marks the area used in the lightness analysis

We measured the average brightness of selected areas as an approximation of the amount of melanin-based skin pigmentation presuming that the lighter the skin, the less melanin there would be (Shriver and Parra 2000; McGraw et al. 2005). We did not use spectrophotometric data in this case because the spectrophotometer probe focuses only a very small spot. The color patterns are more complex and we needed to quantify the pigmentation from the overall area. However, we used the spectrophotometer to check the skin for potential reflectance in UV spectra. No significant reflectance (>5 %) in the UV was detected, therefore we considered the scans to adequately represent the skin lightness as it is perceived.

Selected backs and bellies were converted into grayscale mode, to extract only the brightness information. The area's average brightness was measured with the ImageJ software and displayed as a RGB value. Brightness values were recalculated into percentage by dividing them by 256, which is the total number of steps in the 8-bit RGB brightness scale. Skin melanin pigmentation was represented by two separate values (a) brightness of the back only and (b) averaged brightness of the back and belly, standing for the individual's overall melanin-based body pigmentation.

To evaluate whether the presence of inherent UV reflectance of lingual tissue may be relevant to intra- or interspecific perception, we made an alternative set of spectra with manipulated values. We used sinus function, creating smooth decline in the reflectance curve from the maximum in the human-visible blue range, to the minimal value of the spectrum at 300 nm, therefore depriving the original spectra of the peak in the UV range (Fig. 1).

Visual modeling

We worked with two visual models representing conspecifics and predators. For the representation of conspecifics, we used photoreceptor data for *P. broadleyi*, as those for *Tiliqua* are not known. Diurnal lizards were shown to have a conservative visual system with the retina containing four types of cones, sensitive to UV, short, medium, and long wavelengths (Fleishman et al. 1993; Loew et al. 2002; Whiting et al. 2006; Macedonia et al. 2009), which should apply also to *Tiliqua* (New et al. 2012). *P. broadleyi* belongs to the Scincomorpha clade and hence is the closest relative to *Tiliqua* with available information (Fleishman et al. 2011; Pyron et al. 2013; Perez i de Lanuza et al. 2014b).

Photoreceptor data of *Pavo cristatus* were used for modeling the vision of avian predators (Hart 2002; Ödeen and Håstad 2003). Two types of retina are known in birds, differing in photoreceptors for the shortest wavelengths: an UVS type—with ultraviolet-sensitive cones (peak absorbance under 380 nm), and a VS type, with violet-sensitive cones (peak absorbance above 400 nm) (Endler and Mielke 2005). Birds of prey, but also kookaburras and corvids, have the VS type retina as does the peafowl which is being widely used as a substitute for raptors in visual modeling (Hart 2002; Macedonia et al. 2009; Lind et al. 2013; Ödeen and Håstad 2013; Perez i de Lanuza et al. 2014a).

For calculating chromatic contrasts, we used the TetraColorSpace software (Stoddard and Prum 2008). TetraColorSpace transforms the visual system into a virtual tetrahedral space, following the model of Endler and Mielke (2005), where each vertex represents one of the four photoreceptor types. The perceived color is then projected into the tetrahedron as a point, whose position is calculated from the response of individual photoreceptors to the spectrum and particular illuminant. In our modeling, we have chosen the default illumination set by TetraColorSpace, which represents the standard daylight. The color (chromatic) contrast is then calculated as a Euclidean distance between two points in the color space. Chroma (purity or saturation) of a particular color is expressed as the point's distance from the achromatic origin in the center of the tetrahedron. However, as the maximum distances from the center to the margins are not constant, we counted with the *achieved chroma*, which is the relative distance value. Color contrasts and achieved chroma were calculated for each blue and pink *tongue* (the latter simulated by the oral mucosa) against each of the four background types, for both visual models.

To assess how similar the shades of (1) blue tongues of *T. gigas* and tongues of other skink species and (2) blue tongues of *T. gigas* and UV-blue skin patches of other lizard species look in the eyes of their conspecifics, we calculated color distances, i.e., a chromatic contrast measured in *just noticeable differences* (JND). The calculations for this type of analysis were executed in Avicol v6 (Gomez 2006). The JND measure expresses the discriminability of two colors in a particular visual system, taking into account the receptor noise (Vorobyev and Osorio 1998). A JND value below 1 means that two colors are indistinguishable, a value between 1 and 3 is believed to indicate colors distinguishable only under ideal light conditions, and a value above 3 expresses increasingly distinguishable stimuli (Vorobyev et al. 1998; Siddiqi et al. 2004; Cassey et al. 2009).

First, we calculated color distances for each of the 14 blue-colored tongues to the mean spectrum of *T. gigas*, in order to estimate the individual tongues' intraspecific distances from their overall average. Then we repeated the calculation, this time replacing the average spectrum of blue tongues by a spectrum representing a tongue or a blue skin patch of the selected lizard species. This way we could statistically compare the interspecific distances with the intraspecific ones and thus estimate the proximity of different spectra. We also compared the color distances between UV-positive and UV-negative tongues in both visual models. Input parameters were taken from Marshall and Stevens (2014).

Statistical analyses

The data were tested for normality. The majority of variables had fairly unimodal and symmetric distributions, however, *w* tests revealed multiple cases in which normality was violated at alpha=0.05. Therefore, we selected non-parametric tests instead of parametric ones. We used Spearman's correlation test to detect whether a tinge of the tongue (achieved chroma and/or mean reflectance) is related to other traits. First, we analyzed the individual body size as an approximation of age, expressed as snout-vent length. For the second trait we chose the amount of skin pigmentation, separately for the back and the whole body.

A Mann-Whitney *U* test was used to compare (a) differences in chromatic contrasts of blue and pink *tongues* for each of the four backgrounds and (b) chromatic contrasts of the same color between the two visual models. To assess whether a transition from pink to blue results in greater boost in both chromatic contrast and achieved chroma in one visual model than in the other, we created the following set of data. For each visual model and background, we calculated chromatic contrast differences (dCC) and achieved chroma differences (dAC). dCCs were achieved by subtracting chromatic contrast values of blue tongues from chromatic contrast values of pink *tongues* in all pair-wise combinations and were expressed in absolute values. Similarly, we calculated dACs as differences between *blue* and *pink* achieved chromas. Then, we compared the dCCs (or dACs) between the two visual models using the Mann-Whitney *U* test.

We used a Kruskal-Wallis test to detect significant differences within the set of intra- and interspecific color distances. When the results proved to be significant, a Mann-Whitney *U* test was applied to compare the individual pairs of data; intraspecific color distances for blue tongues were each time put against color distances between tongues of *T. gigas* and tongues (or UV-blue skin samples, respectively) of other lizard species.

We performed a Wilcoxon matched pair test to compare the color distances between UV-positive and UV-negative tongue spectra within both visual models. All analyses were executed in Statistica 8 (StatSoft, Inc. 2007).

Results

Our spectrophotometric measurements showed that both blue tongues and pink oral mucosa share very similar spectral shape, differing notably in longer wavelengths. Both spectra have primary maximum peak in the UV range at 320 nm and secondary, a slightly lower one, in the blue range, around 460 nm (Fig. 1).

The Spearman's test did not reveal any significant correlations between color qualities of the tongue and selected traits; compared against snout-vent length: for tongue lightness $r_{\mathrm{Spearman}}=-0.036$ ($P=0.901$); for achieved chroma of the tongue $r_{\mathrm{Spearman}}=-0.493$ ($P=0.073$); compared against pigmentation of the back: for tongue lightness $r_{\mathrm{Spearman}}=0.461$ ($P=0.083$); for achieved chroma of the tongue $r_{\mathrm{Spearman}}=0.438$ ($P=0.102$); compared against the overall body pigmentation: for tongue lightness $r_{\mathrm{Spearman}}=0.354$ ($P=0.196$); for achieved chroma of the tongue $r_{\mathrm{Spearman}}=0.232$ ($P=0.405$).

The Mann-Whitney test indicated higher chromatic contrasts of blue tongues compared to the pink *tongues*; for the conspecifics visual model, it was significant against light brown (Z=-2.80, $P=0.005$), dark brown ($Z=-2.85$, $P=0.004$), orange-reddish ($Z=-2.80$, $P=0.005$) and grayish backgrounds ($Z=-2.85$, $P=0.004$); for the avian predator visual model, it was significant against light brown ($Z=-3.86$, $P<0.001$), dark brown ($Z=-3.72$, $P<0.001$), orange-reddish ($Z=-3.86$, $P<0.001$) and grayish backgrounds ($Z=-3.86$, $P<0.001$) as well. At the same time, blue tongues were more contrasting against light brown ($Z=2.71$, $P=0.006$), dark brown (2.94, $P=0.003$) and grayish backgrounds ($Z=3.54$, $P<0.001$) in the visual model of conspecifics than in the predator one. Pink *tongues* were significantly more contrasting against light brown ($Z=2.34$, $P=0.019$) and grayish backgrounds ($Z=2.71$, $P=0.007$) in the conspecifics visual model (Fig. 3).

Blue tongues had also higher values of achieved chroma compared to pink *tongues* in both the conspecifics visual model (blue=0.290±SE 0.014, pink=0.192±SE 0.021; $Z=-3.492$, $P<0.001$) and the avian predator visual model (blue=0.271±SE 0.015, pink=0.173±SE 0.010; $Z=-3.583$, $P<0.001$). The difference between pink and blue chromatic contrasts (dCC), and pink and blue achieved chromas (dAC) respectively, was statistically insignificant when the two visual models were compared ($P>0.05$).

The mean intraspecific color distance of *T. gigas*' tongues from their average was 1.57 JND±SE 0.42 (minimum=0.12 JND, maximum=4.96 JND). Overall interspecific differences were significant: for tongues, Kruskal-Wallis test: $H=38.9$, $P<0.001$, and for blue tongues versus UV-blue skin, Kruskal-Wallis test: $H=84.1$, $P<0.001$. The tongue of *C. gerrardii* (2.13 JND±SE 0.34; $Z=1.42$, $P=0.154$) and the shoulder spot of our captive *G. galloti galloti* (1.68 JND±SE 0.34; $Z=0.50$, $P=0.613$) had the closest shades to the tongue of *T. gigas*, with color distances statistically corresponding with its intraspecific span of shades. The second closest hue was measured for the ventrum of *P. lilfordii kuligae*, (2.89 JND±SE 0.34; $Z=-2.39$, $P=0.017$) which fell also into the transitional 1–3 JND range (Fig. 4).

The comparison of UV-positive and UV-negative tongue spectra revealed that they are perceived as substantially distinct colors in the eyes of conspecifics (10.45 JND±SE 0.25), while being hardly distinguishable by avian predators (1.79 JND±SE 0.09; $Z=5.16$, $P<0.001$).

Discussion

We explored the visual qualities of blue lingual surface in the blue-tongued skink *T. gigas* to compare it with similar semantic signals of other lizard species. We have demonstrated that the blue tongue expresses a significant reflectance in the UV

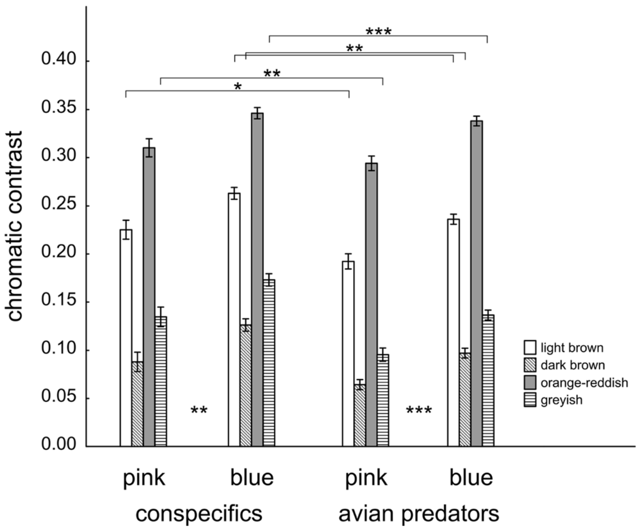

Fig. 3 Mean values of chromatic contrasts of blue tongues and pink *tongues* against four different background colors. *Asterisks* stand for significance of the Mann-Whitney *U* test (those situated between the column sets relate to differences between corresponding values within the visual model): *$P<0.05$, **$P<0.01$, ***$P<0.001$. *Bars* indicate ±SE

spectrum and a higher color contrast against various backgrounds (especially for the vision of conspecifics). The UV-blue lingual color as well as UV-blue patches of other lizard species fit into the same UV-sensitive visual communication channel of diurnal lizards. This leads us to the assumption that the blue tongue coloration might play an intraspecific signaling role.

The reflectance curve of pink oral mucosa, including the primary peak in the UV range, is obviously a general character of bare tissue, as it corresponds with the spectral reflectance of an open mouth and unfeathered skin of birds' nestlings (Hunt et al. 2003; Jourdie et al. 2004). Pink is not a spectral color and arises from combining two reflectance peaks instead of having just one (Burkhardt 1989; Endler 1990). As reflectance curves of blue tongues match the same shape with the exception of the longer wavelengths part, it is likely that a certain degree of lingual pigmentation suppresses the *red* peak, while retaining the *blue* peak, which leads to the blue coloration.

The shift from unpigmented to pigmented blue tongues notably leads to their higher conspicuousness, especially in their chroma and chromatic contrasts. Although the degree of change between pink and blue is virtually the same, blue was generally more contrasting in the conspecifics model than in the avian predator model. Taking also into account the results of UV-positive and UV-negative spectra comparison, we may assume that blue tongues are more likely to be adjusted to the sight of skinks, rather than to avian predators, which also corresponds to their photoreceptors sensitivity (UVS versus VS cones). This is in accordance with several studies suggesting that UV signs hardly cause any specific response in raptors, which gives their prey the advantage of using the ultraviolet communication channel (Endler 1992; Håstad et al. 2005; Lind et al. 2013; Marshall and Stevens 2014). Neither would be UV likely to work as an aposematic signal to birds with a UVS vision (Lyytinen et al. 2001). Apart from birds, *Tiliqua* are preyed upon by a range of terrestrial predators, but there is even more ambiguity regarding whether some of those can be directly associated with the evolution of lingual coloration.

Elapids and monitor lizards are possible candidates for predators putting *Tiliqua* skinks under evolutionary pressure (Shine and Keogh 1996; Fitzsimons 2011, Fleay 1950 ex Mayes et al. 2005), but little is known about their visual

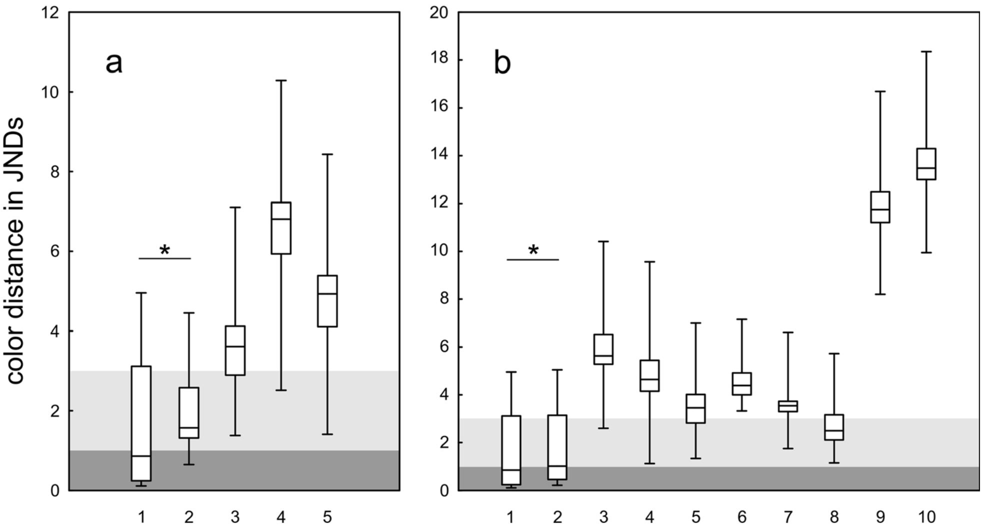

Fig. 4 Color distances of 14 blue tongues of *T. gigas* from mean values of tongues (**a**) and UV-blue skin patches (**b**) of selected species. **a** *1 T. gigas*, *2 C. gerrardii*, *3 E. frerei*, *4 C. zebrata*, *5 T. perrotetii*. **b** *1 T. gigas* (tongue), *2 G. galloti galloti*, captive individual (shoulder spots), *3 G. galloti galloti* (shoulder spots), *4 G. galloti eisentrauti* (shoulder spots), *5 T. lepidus* (lateral eyespots), *6 D. caucasica* (OVS), *7 P. pityusensis* (OVS), *8 P. lilfordi kuligae* (ventrum), *9 P. lilfordi kuligae* (OVS), *10 P. broadleyi* (throat). *Mid-line* median, *box* 25-75 %, *whiskers* minimum-maximum, *dark gray area* less than 1 JND, *light gray area* 1–3 JNDs, *$P>0.05$ (Mann–Whitney *U* test)

systems. A study on hydrophiid sea snakes (Hart et al. 2012) shows they are trichromatic and lack UV-sensitive cones, which can be however associated with their transition into the sea, while terrestrial elapids may still have UVS cones preserved as in other snakes (Sillman et al. 1997, 1999, 2001; Macedonia et al. 2009). Monitor lizards have not been examined in this respect so far, but being diurnal, it is likely they are tetrachromatic and UV sensitive like other lizard groups (Perez i de Lanuza et al. 2014b). It is also worth mentioning that marsupials have UV-sensitive cones as well (Deeb 2010), so the UV-reflective tongues could be an appropriate stimulus for their vision too. Recent carnivorous marsupials (belonging to the Dasyuromorphia group) are, however, mostly nocturnal (Miller and Herbert 2010) and therefore these potential predators are unlikely to interact with diurnal *Tiliqua* and to present a serious selective pressure.

If the lingual coloration presents an anti-predation signal, UV-sensitive reptilian predators would be the target recipients more likely than birds. Bustard (1964) gives an account on such defensive gaping reaction of a gecko *Strophurus williamsi* towards a larger gecko of the genus *Oedura* (geckos can be also UV sensitive; Loew 1994; Loew et al. 1996). Like *Tiliqua*, geckos of the genus *Strophurus* (tribe Diplodactylini) have conspicuously colored blue/black or less frequently orange/yellow tongues and mouth interiors, which are exposed during defensive displays (Bustard 1964; Melville et al. 2004). Based on phylogenetic data, Melville et al. (2004) conclude that while pink mouth is ancestral, striking mouth colors may have evolved with a transition of *Strophurus* geckos to diurnal activity. The pattern in *Tiliqua* and its relatives is not so unambiguous. Although both large skinks with pink tongues, *C. gerrardii* and *C. zebrata* are reported to be crepuscular to nocturnal (Mann and Meek 2004; Cogger 2014), there is a number of other crepuscular and/or nocturnal species which do have bluish or dark tongues and mouths; *Liopholis kintorei*, *Cyclodomorphus branchialis*, *Cyclodomorphus casuarina*e, etc. (Shea and Miller 1995; Chapple 2003). Thus, even though blue tongues and/or mouths are a visual signal in both *Tiliqua* and *Strophurus*, diurnal activity is a precondition rather than a trigger for evolution of such trait. Based on the phylogeny by Pyron et al. (2013), the distribution of pigmented tongues rather suggests that they were already present in the common ancestor of the *Egernia*-*Liopholis*-*Cyclodomorphus*-*Tiliqua* group.

The lingual pigmentation itself forms rather a black–blue continuum, which can be noticed on the spectral curves (the lower the shape, the darker the tongue; Fig. 2), but also on their projection into the tetrahedral color space (Fig. 5). Dark pigmented tongues of *E. frerei* and *T. perrotetii* and even a pink tongue of *C. zebrata* lay at the lower end of a color space occupied by the blue tongues (closer to the achromatic center of the tetrahedron), which indicates similar hue, but different lightness and saturation. Their relative proximity is caused mostly by the common dominant reflectance in short wavelengths. Minimum color distance between tongues of *T. gigas* and *E. frerei* (or *T. perrotetii*) was only around 1.4 JND, so there is a certain overlap in the span of chromatic distances—some *T. gigas* have their tongues more similar in color to other species than to some of their conspecifics. The tongue closest in color belonged to *C. gerrardii*, which represents a peculiar case among blue-tongued skinks. Juveniles have blue tongues, but the pigmentation disappears with age and adults have their tongues mostly pink, though—as in our case—some remnants of pigmentation may still remain (Brown 2012).

Similar situation results from the comparison of tongues with UV-blue skin patches. The shade being the worst distinguishable from the blue tongues was the shoulder spot of our single captive individual of *G. galloti galloti*. Data on a much larger sample of wild *G. galloti* from literature resulted however in significantly greater color distances. As our experience with captive and wild animals shows, lizards may partially lose the intensity of their coloration in captivity, but intraspecific variation can be taken into account as well. In addition, the second closest hue was the blue ventrum of *P. lilfordi kuligae*, with the color distance also within the transitional range between indistinguishable and clearly distinguishable colors (1–3 JNDs).

Although we found only two occasions when a tongue or a skin patch had a color statistically interchangeable with the tongue of *T. gigas*, one should keep in mind that the spectra of other species were represented mostly by mean values which would be otherwise surrounded by clouds of raw data points. So at least some partial interspecific overlaps in color spaces can be expected. Since the color space occupied by the blue tongues is surrounded by spectra of UV-blue skin patches, tongues of *T. gigas* may possibly have qualities of a semantic signal, analogous to that of other lizards.

Co-evolution of visual signals with visual systems and behavior has been demonstrated in lizards (Fleishman et al. 2011; Landová et al. 2013; Martin et al. 2015), therefore it is likely that tongue pigmentation may represent such a case and serve in intraspecific communication. Although the skinks of the genus *Tiliqua* are closely related to the *Egernia* group of skinks, characterized by their social behavior and long-term relationships between individuals (Chapple 2003; Main and Bull 1996; Gardner et al. 2008; Fenner et al. 2012), little is known about the social organization of typical blue-tongued skinks of the *scincoides-gigas* group (Price-Rees et al. 2012, 2014; Pyron et al. 2013). Price-Rees et al. (2012) reported that there was no mutual influence of one lizard's location to another, which would suggest low importance of *direct behavioral interactions in these high-density core areas*. However, their study was based on radiotelemetric analyses, which does not allow detection of any visual communication between the lizards. So the putative lack of direct interactions may just as well have been caused by a specific visual signal. Due to

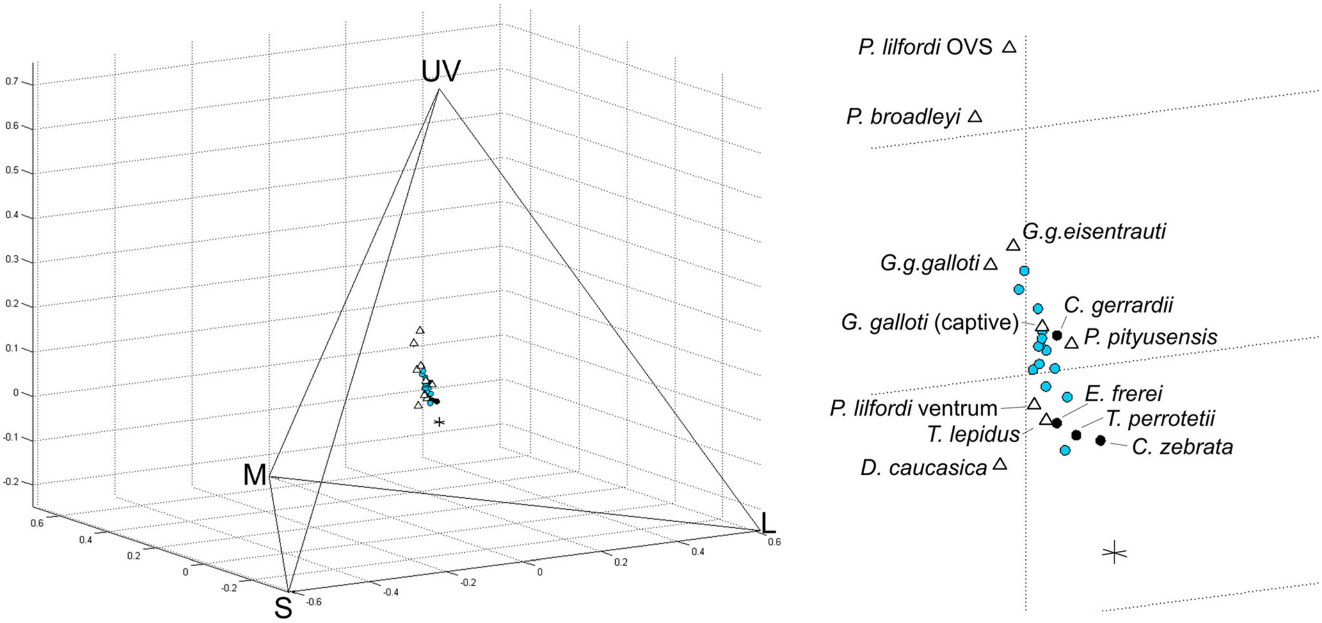

Fig. 5 Visual model of conspecifics with the distribution of analyzed spectra in the tetrahedral color space. Blue tongues of *T. gigas* (*shaded circles*; *n*=14), mean values for tongues (*black circles*), mean values for UV-blue skin patches (*triangles*). *Cross mark* indicates the achromatic center of the color space. *Vertices of the tetrahedron* stand for maximum stimulation values of photoreceptors sensitive to long (*L*), middle (*M*), short (*S*), and ultraviolet (*UV*) wavelengths

considerable aggressiveness of *Tiliqua*, there might be a need to avoid a potential conflict. Murray and Bull (2004), for instance, report higher aggressiveness of *T. rugosa* males towards conspecifics than non-conspecifics and Langkilde and Shine (2005) observed that open-mouth threat often preceded biting in *Egernia*. The tongue coloration therefore may possibly improve the signaling effect.

Our results have not confirmed that the darker the individual, the darker or bluer its tongue. Unless this was caused by a low sample size, we can assume that the intensity of lingual coloration is not much associated with the amount of skin melanin pigmentation, but can still represent an honest signal correlated with other factors like health, endurance, hormone levels, or age. According to our calculations the correlation coefficient suggests possible negative correlation between the body size and tongue saturation, although the result has not proved to be significant. Several other factors, like a growth rate, population, and larger sample size including also juveniles and subadults would give a more accurate picture of this relationship. In any case, even if lingual coloration was independent on the above-mentioned factors, this still would not exclude its possible signaling or recognition function.

Blue-colored tongues may enhance the open-mouth threat during interspecific interactions, and can be also secondarily used as an anti-predatory response which would probably be the most effective towards reptilian antagonists. Yet the tongue coloration need not be involved in close encounters only, but can also boost inter-individual recognition at longer distance interactions. Effective recognition of potential aggressors at distance, where the risk of threat is not immediate, may be of certain importance in avoiding conflicts. We suggest further behavioral tests to be made to clarify interspecific interactions of the blue-tongued skinks, the impact of tongue display on their aggression and predation and to examine possible correlations between tongue coloration and physiological aspects like endurance or hormone levels.

Acknowledgements We thank Leo Fleishman for kindly providing photoreceptor data for *P. broadleyi*, Tomáš Grim and Dan Hanley for lending the spectrophotometer, Veronika Holáňová (Hříbalová) and Tomáš Protiva for providing *C. zebrata* and *C. gerrardii*. The work was supported by Grant Agency of Charles University in Prague (project No. 754213).

Ethical standards This study was allowed by Ethical Committee of the Charles University in Prague, Czech Republic and approved by Ethical Committee of Ministry of Education, Youth and Sports, license no. 26582/2012-30.

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II

Ultraviolet reflectance and pattern properties in Leopard geckos (*Eublepharis macularius*)

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Submitted to Behavioral Ecology and Sociobiology

Ultraviolet reflectance and pattern properties in Leopard geckos (*Eublepharis macularius*)

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Acknowledgements

We would like to thank to Michal Šulc for spectrophotometric measurements of our calibration standards and Kristýna Jachnická for help with taking pictures. We are grateful to Petra Chumová, Kristina Hlaváčová, Tereza Fictumová and Eliška Kočková-Amortová for taking care for animals. This work was supported by the Grant Agency of Charles University (No. 1636218/2018) and SVV project 260434/2019. Participation of P. F. was supported by the Charles University Research Centre program No. 204069.

Abstract

Complex visual signaling through various combinations of colors and patterns has been well documented in a number of diurnal reptiles. However, there are many nocturnal species with highly sensitive vision, being able to discriminate colors in night conditions, as was shown in geckos. Because of their sensitivity to chromatic signals, including UV, they may have potential hidden features in their coloration, which may play role in intraspecific communication (e.g. mate choice) or interspecific signals (e.g. antipredatory function). We explored this hypothesis in nocturnal Leopard geckos (*Eublepharis macularius*), a species using visual signals in both antipredation defense and courtship, having ontogenetic color change accompanied by a shift in behavior. We used UV photography and visual modelling in order to compare various aspects of their coloration (luminance, contrast, color proportions) between sexes, age groups and populations. We found that Leopard geckos have considerable UV reflectance in white patches on their tails (and on the head in juveniles). Though, no prominent differences were detected in their coloration between various groups. We hypothesize that the limitation of UV reflectance to the head and tail, which are both actively displayed during defense, especially in juveniles, might potentially boost the effect of antipredation signaling.

Significance statement

Color signals were studied mostly in diurnal animals, however, there is also a number of visually oriented nocturnal species, some having even color night vision. Besides, many animals see ultraviolet light, which serves in intraspecific communication. Both conditions – color night vision and UV vision – occur in geckos. We detected the presence of ultraviolet markings in Leopard geckos and used visual models to explore whether those markings express any signs of adaptive traits, e.g. sexual dimorphism, or ontogenetic change, as potential drivers for the development of such traits exist in Leopard geckos: age-dependent antipredation behavior and strong male-male competition. Our study found no dimorphism in UV traits. However, the UV component is found always on the tail, which is frequently exposed during antipredation defense and intraspecific communication. Therefore, we hypothesize that UV may contribute to the overall conspicuousness of this organ and more effective signaling, respectively.

Keywords: UV – visual signaling – antipredation – Squamata – visual modeling

Introduction

Colors and patterns play a significant role in the lives of many species of reptiles, contributing to both intra- and interspecific communication, spanning from warning signals and mimicry (aposematic snakes, *Heliobolus* juveniles) through courtship displays (anoles, sitanas) and honest signals (*Platysaurus*, *Uta*) to crypsis or even to combinations of more than one strategy (chameleons, *Diadophis*; for review see Olsson et al. 2013). As many reptile species have also ultraviolet-sensitive cones, they use UV coloration in their signaling as well (Fleishman et al. 1993; Loew et al. 2002). Usually, it is involved in sexual selection. In reptiles, the mate choice based on coloration was observed in a number of species, e.g. females of European green lizards (*Lacerta viridis)* prefer males with high UV throat reflectance (Bajer et al. 2010), females of the Eastern fence lizard (*Sceloporus undulatus)* prefer males having bigger throat badges, which signal their quality (Swierk et al. 2012), in some populations of Common chuckwalla (*Suromalus obesus)* and Common collared lizard (*Crotaphytus collaris)* color-based female choice occurs as well (Kwiatkowski and Sullivan 2002; Baird et al. 1997). However, some studies questioned the importance of female choice in lizards and attribute the special coloration of males to the effect of increased male-male competition (LeBas and Marshall 2001). The greater fighting ability of Augrabies flat lizards (*Platysaurus broadleyi*) is associated with more intense UV coloration of the throat (Whiting et al. 2003, Stapley and Whiting 2006, Whiting et al. 2006).

Of course, all those cases are found in diurnal lizards. Yet, UV sensitive cones have been confirmed also in geckos, which are typically nocturnal. Their vision is adapted to low light conditions in several ways, including large eyes, photoreceptor morphology or absence of fovea (Röll 2001). Despite being active during the night, geckos have retained color vision inherited from their diurnal ancestors (Gamble et al. 2015) and have been shown to discriminate colors even in very dark conditions, as was documented in *Tarentola chazaliae* (Roth and Kelber 2004). While most nocturnal animals have rod-dominated retina, which is highly sensitive to brightness, but not to colors, geckos have all-cone retina, which suggests they evolved from tetrachromatic ancestors, subsequently losing the LWS photoreceptor (Röll 2000), but retaining the ability to switch back to diurnal activity, which happened in several genera, e.g. *Phelsuma, Naultinus, Lygodactylus, Gonatodes, Rhoptropus* etc. (Gamble et al. 2015). The microscope and micro spectrophotometric examinations were conducted for a number of species from the related families Gekkonidae and Sphaerodactylidae, including *Hemidactylus turcicus, H. garnotii, Teratoscincus scincus* (Loew et al. 1996), *Gekko gecko* (Crescitelli et al. 1977) or *Gonatodes albogularis* (Ellingson et al. 1995). Current evidence shows that geckos are trichromatic, possessing UVS, SWS and MWS cones, with their maximum sensitivities around 364, 460 and 525 nm, respectively.

In this study, we decided to find out whether a nocturnal reptile with a high probability of having color night vision possesses visual traits, either in UV or visible spectrum, which could play part in within or between species signaling. For this purpose, we chose Leopard geckos (*Eublepharis macularius*), a widely used reptilian model organism in various fields of research because of their relatively easy keeping, breeding, larger size and advanced behavior. Although vision of eublepharid geckos has not been studied so far, the family Eublepharidae is phylogenetically close to (it branches off just before) the group containing the families Sphaerodactylidae and Gekkonidae (Gamble et al. 2015), both of them represented by trichromatic species with UVS cones (Crescitelli et al. 1977; Ellingson et al. 1995; Loew et al. 2002). Therefore, we suppose that Leopard geckos are candidates for color night vision as well.

Leopard geckos are crepuscular to nocturnal animals inhabiting rocky landscapes with low vegetation from Afghanistan to India. They can be found under stones, tree bark and even in the vicinity of human settlements. Males are territorial and aggressive during the reproductive period. Their diet consists of various invertebrates, smaller reptiles or new-born rodents. Coloration is highly variable but basically consists of black spots on yellow background with grey and white bands on the tail. The ventral side is uniformly white (Szczerbak and Golubev 1986; Bradley and Nieves 1999; Khan 2006).

Leopard geckos are remarkable for the change in coloration during their ontogeny accompanied by a change in their behavior. The dorsal pattern in juveniles is characterized by contrasting yellow, black and white bands, which gradually disintegrate into spotty, cryptic pattern typical for the adults. Simultaneously, their antipredation strategy shifts from taking a defensive posture and vocalizing in juveniles, to escaping in the adults (Landová et al. 2013). When aroused or alert, they slowly wave their tails up in the air. The slow tail waving is a part of high (in juveniles) as well as low (in subadults) antipredator postures when the geckos are facing the living snakes (Landová et al. 2016). Potential optical signals in UV could strengthen this antipredator tactic when the predator attention is redirected from the body to the tail (Congdon et al. 1974, Cooper and Vitt 1985). The tail can be easily autotomized in this species (Higham et al. 2013).

Besides antipredation, optical communication also works together with chemical communication during the courtship (reviewed in Mason and Parker 2010). Males of the Leopard gecko routinely lick all individuals they meet. If the encountered animal is a female in breeding condition, the male starts his courting (Mason and Gutzke 1990) with stilting postures, usually accompanied by tail vibrations (LaDage and Ferkin 2006). Those are expressed only in the courting context in this species (Brillet 1993). However, the behavior of females is essential for successful mating in this species. Initially, the female observes the courting behavior of a male. A receptive female remains stationary when contacted by a courting male (Gutzke and Crews 1988) and if she decides to accept his courting, she further allows the male to approach her. On the other hand, if the female is not receptive or decides to refuse the male, she will terminate the courting by fleeing or biting. Therefore, the male coloration, especially on the tail, could be an important signal for female choice during the courtship. The courting behavior was repeatedly observed not only at night, but also during the day (EL, PF). As was shown in birds, strong sexual selection on males has antagonistic effects on each sex, increasing the colorfulness in males, while reducing it in females (Dale et al. 2015). As the intraspecific aggression between males of Leopard geckos exists (Kratochvíl and Frynta 2002), we can assume that at least some chromatic signals including UV can serve as a condition-dependent trait reliably reflecting individual phenotypic quality. The complex color pattern (including UV) of the Leopard gecko should be semantic in two basic contexts: 1) antipredator defense that undergoes apparent change between juveniles and adults and 2) intraspecific signaling between adult males or between opposite sexes, both cases being important for sexual selection and mirrored by sex differences in color pattern.

In this paper, we explore the coloration and pattern of Leopard geckos in the UV-visible range (300-700 nm). Generally, we search for differences between age groups, sexes and populations and discuss their potential biological relevance in the context of antipredator behavior and sexual selection. Our particular aims were 1) to compare the luminance and proportions of certain colors between juveniles, subadults and adults in order to explore whether the change in color pattern follows the changes in antipredator strategies that have been demonstrated previously (Landová et al. 2013). We wonder if the proactive defense of juveniles is associated with more apparent appearance including more intense UV signal compared to adults, which have escaping tactics more prevalent. 2) To compare the coloration parameters between sexes, predicting that intense female choice and undergoing sexual selection will be associated with pronounced sexual dimorphism in coloration (Dale et al. 2015).

Material and methods

Animals

We examined 236 leopard geckos (*Eublepharis macularius*) kept in our faculty facilities. The sample consisted of 133 adult females, 65 adult males and 38 juveniles. 188 animals (44 males, 106 females, 38 juveniles) belonged to a group descended from a wild population in Pakistan, being highly variable in color pattern and morphology (Jančúchová-Lásková et al. 2015) and growth rate (Frynta et al. 2018). The subpopulation used here is the first, second and third generation and further referred to as 'yellow' (for the details about the origin of our laboratory stock see Starostová et al. 2005; Frynta et al. 2018). The remaining individuals (21 males, 27 females) belonged to a laboratory strain (henceforth 'lab') bred in captivity since the 1970s in the Czech Republic. As mentioned above, this species undergoes color change during its ontogeny. We defined the age categories according to Landová et al. (2013): juveniles (striped pattern) 0-90 days old, subadults (adult-like pattern) 90-450 days old, and adults (fully disruptive coloration) more than 450 days old.

The studied species has temperature-dependent sex determination (Viets et al. 1993), and the incubation temperature may also affect behavioral traits of these animals (cf. Flores et al. 1994; Sakata and Crews 2004). To avoid the possible bias towards hatching non-territorial females, we set the incubation temperature to 28.5 °C±0.5, which is close to the temperature (approximately 29 °C) preferred by females of *E. macularius* for egg laying (Bull et al. 1988; Bragg et al. 2000).

The ambient temperature of the breeding room was about 28 °C with the permanent presence of basking cables under each terrarium to maintain a temperature gradient. The juveniles were housed singly in plastic boxes 20×20×15 cm, and adults were housed in glass terrariums 30×30×20 cm. Food and water were provided ad libitum. Hatchlings were fed with crickets; since 3 months of their age, the diet was supplemented by mealworms (cf. Gauthier and Lesbarrères 2010). The insects were dusted with vitamins and minerals (Nutri Mix); $AD_3$and E vitamins were provided weekly.

Image processing

Each individual was photographed from the top in both visible and UV spectra (Fig. 1a-d). For this purpose, we followed the methodology of Troscianko and Stevens (2015). We used a specially adapted digital photo camera

Samsung NX1000 with removed UV/IR blocking filter, and 35mm lens Novoflex Noflexar. As a light source, we used Iwasaki eyeArcColor MT70D (70W E27 6500K) with removed UV-protecting coating. The bulb was set about 40 cm above a white teflon (PTFE) arena, where the geckos were placed. Each individual was photographed in a shaded room at a setting ISO400, F/16, through both Baader UV-IR-cut filter, transmitting visible light (400 to 700 nm), and Baader Venus-U filter, transmitting UV light (300 to 400 nm). A PTFE tape stretched around a PTFE strip in 10 layers was used as a calibration standard. Its reflectance was checked with a JAZ Ocean Optics spectrophotometer against a WS-1 white standard and was estimated to be 99%.

The adults were photographed twice within a period of 2-9 months. The juveniles were followed from their hatching for the next 11 months and the photos were taken approximately every 2-4 weeks.

Pattern and luminance analyses

Photos in a RAW format were processed using a Multispectral Image Calibration and Analysis Toolbox (MICA) (Troscianko and Stevens 2015), a plugin operating within ImageJ software (Schneider et al. 2012). We measured luminance (perceived brightness) of white, grey and black colors on the tail of every individual. Three patches of each color were selected on the tail (Fig. 2a). Grey color is absent in the hatchlings and gradually emerges from the center of the black bands, so its measurement began as soon as the new shade was detectable. In the juveniles, we measured – apart from the tail – also the white crescent on their head, as both regions are displayed when gaping and tail waving during their defense (Landová et al. 2013) (Fig. 2b). In adults, we further selected both the whole tail and the whole back for analyses of pattern and contrast.

The software processes the pattern information by filtering the image at different spatial frequencies through fast Fourier transformation, using standard deviations of pixel values to calculate ‘pattern energy’ (Bex and Makous 2007; Chiao et al. 2009; Stoddard and Stevens 2010). Our setting started on 2px and continued by multiples of √2 to 512px. All images were downscaled to 17px/mm. We retrieved the following variables: mean luminance, maximum frequency (the spatial frequency with maximum energy, a measure of the dominant markings’ size), total energy (sum of energies on all spatial frequencies, a measure of pattern contrast) and a proportion of pixels according to their luminance. We quantified the extent of UV reflective patches on geckos’ backs, defined as areas having at least 8% reflectance in the UV channel (as both black and yellow pigmented areas reflected less than 8% of UV light in at least 95% of cases).

The multispectral images were converted to cone catches of a trichromatic gecko *Hemidactylus garnotii*. Despite the fact, that the models are designed for the daylight and (photopic) vision, while the Leopard geckos are mostly nocturnal, we chose this option because it is the closest biologically relevant approximation we can currently achieve. We used the medium wave sensitive photoreceptor for the luminance (achromatic) channel (Spottiswoode and Stevens 2010). We also recorded the response of the UVS photoreceptor in order to explore luminance in the UV spectrum.

To quantify proportions of individual colors, we used Barvocuc software (Rádlová et al. 2016), which measures the percentage of the area they occupy. First, false-UV color images were created with the MICA Toolbox, to take account of the UV component in the overall coloration. The area of interest included the whole dorsal side of a gecko except its limbs to filter out potential artefacts caused by their highly variable positions during the shooting (Fig. 2c). As both white and black gradually become grey during geckos' ontogenesis and a strict line cannot be drawn between them, we treated white and grey areas together (both being UV reflective colors), while black and yellow were treated separately.

Statistical analysis

STATISTICA 8 (StatSoft, Inc. 2001) and R project (R Development Core Team 2008) with 'nlme' library (Pinheiro et al. 2019) were used for statistical analyses. 'Yellow and 'lab' populations were treated separately. Variables were checked for normality and log-transformed if needed. Arcsine transformation was applied to percentages of color areas to stabilize the variance and normalize proportional data. Sex and population differences were tested with both discriminant analysis and t-test. White patches on the head and the tail in the juveniles and subadults were compared with paired t-test for every month of age. Differences between the three age groups (juveniles, subadults, adults) were checked with a GLS method with compound symmetry correlation structure and 'individual' as a random factor, to correct the effect of repeated measurements. Bonferroni correction was applied where necessary. The effect size was estimated with Hedge's g, which compares the distance of sample means, taking into account their standard deviation weighted by the sample size.

Results

Photographic evidence confirmed considerable UV reflectance in white and grey region (8-35%), while both black and yellow regions absorbed UV light (maximum reflectance 6% and 8%, respectively). The discriminant analysis was unable to distinguish between the sexes in either population by any of the traits examined (Wilks' lambda: 'yellow' 0.68-0.70, 'lab' 0.43-0.48; $p>0.05$). Partial t-tests detected small, but statistically significant differences in certain traits in the 'yellow' population. On average, males had higher luminance of the white patches on the tail ($t = 3.130$, $p = 0.002$, $g = 0.57$), mean luminance of the tail ($t = 2.990$, $p = 0.003$, $g = 0.53$), total 'energy' (contrast) of the tail ($t = 2.994$, $p = 0.004$, $g = 0.53$), mean luminance of the back ($t = 3.801$, $p < 0.001$, $g = 0.68$) and proportion of yellow and black coloration, respectively (yellow: $t = 4.607$, $p < 0.001$, $g = 0.82$; black: $t = -3.357$, $p = 0.001$, $g = -0.60$) (Fig. 3). In absolute values, the means differed by a few per cent at most (Table 1). Predominant marking size peaked at the same spatial frequency in both sexes. In the 'lab' population, the difference was statistically insignificant in all cases. No significant difference was detected in the UV range.

Paired t-test, comparing the luminance of the white patches on juveniles' head with those on the tail, revealed significant difference only for the fifth ($t = 3.529$, $p = 0.003$) and sixth month of age ($t = 3.331$, $p = 0.003$), with head being paler by 3.5% to 4%, respectively.

According to GLS test, the luminance of white areas remained basically constant throughout the life (visible: $F = 2.628$, $p = 0.073$; UV: $F = 2.215$, $p = 0.11$), while the luminance of grey significantly differed between age groups (increased with age) in both visible ($F = 99.198$, $p < 0.001$) and UV spectra ($F = 86.976$, $p < 0.001$) (Fig. 4a). Color proportions of the dorsal pattern came out as age dependent: black ($F = 84.234$, $p < 0.001$), yellow ($F = 124.699$, $p < 0.001$), white+grey ($F = 131.838$, $p < 0.001$). All three colors have approximately the same proportions in juveniles. In the subadults, black color retreats due to emergence of grey. In adults, yellow color dominates, followed by black, while the least area is covered by white and grey (Fig. 4b).

Discussion

Our results show that those areas in Leopard gecko coloration, which lack pigmentation, reflect considerable amounts of ultraviolet light. Szydłowski et al. (2016) confirmed the presence of xanthophores, containing granules of carotenoid or pteridine pigments, and melanophores in Leopard geckos, responsible for yellow and black coloration, respectively. They also found no pigmentation in the white areas, including the belly. As the UV reflectance is restricted mainly to pigment deficient skin patches, we assume that UV reflectance is caused simultaneously by the absence of pigmentation and by the microstructure of the skin or deeper tissue.

The fact that the UV reflective white regions are limited to the tail (at all ages) and the head (in juveniles and subadults) may suggest some biological relevance. Conspicuous tails are typical for juveniles in a number of lizard genera (*Podarcis*, *Eumeces* etc.) and their likely function is to deflect predator's attack to the least vulnerable part of the body and/or to prevent the larger conspecifics from attacking the youngs (Congdon et al. 1974; Cooper and Vitt 1985; Hawlena 2009). This could be of certain importance in Leopard geckos, as cannibalism was reported in their youngs (Bonke et al. 2011). Antipredation behavior in juveniles and subadults of the Leopard gecko includes deterrent vocalization often accompanied by tail waving. Adults also slowly wave their tails, with their bodies stuck to the ground when they sense a snake in their vicinity (Landová et al. 2013, 2016). The tail may, therefore, serve as a deflector. The phenotype of the subadults can be viewed as an intermediate stage between the juveniles and the adults, with one type of antipredation strategy replacing another. However, this transitional phase, when both defensive reactions are present can be also viewed as adaptation. The subadults represent the palest stage (due to pattern rearrangements) in the life of Leopard geckos. This potentially makes them most conspicuous and vulnerable, therefore it is not surprising that the escape gradually becomes the dominating reaction (Landová et al. 2013), but both antipredation strategies are still present, which can be advantageous.

The situation in juveniles seemed to be less obvious because of their overall banded pattern, which makes them conspicuous as a whole. Though, as our results showed, the white bands – unlike the yellow ones – reflect UV light, which may add more contrast to the tail pattern and make the stripes look white for UV-sensitive animals too (Cronin and Bok 2016). The UV-white crescent, covering the neck and upper lips in juveniles, highlights the head, which somewhat contradicts the 'deflector tail' hypothesis. However, juvenile geckos often open their mouths and vocalize in defense. The head stripe may boost the gape warning and therefore the overall contrasting pattern can function rather as a complex startling signal, having aspects of both aposematic and disruptive coloration.

Taking into account the crepuscular to the nocturnal activity of Leopard geckos, UV reflective stripes may appear even more dominant during the twilight, when the relative amount of UV irradiance increases (Palmer and Johnsen 2015; Spitschan et al. 2016). And although only small quanta of UV reach the Earth's surface in the night, short-wavelength photons have relatively higher energy and stronger scattering than the long wavelengths (Stover 1995). Therefore, we cannot rule out that UV reflective patterns might be advantageous even for nocturnal animals.

Considering pattern and coloration, our study found no systematic inter-group differences in the intensity or distribution of the UV cues. Little, but significant differences were detected in the visible spectrum (perceived luminance) in the ‘yellow’ population of Leopard Gecko. The most significant was the proportion between black and yellow coloration; males appeared to have more yellow, while females more of black colored areas. This can be explained in terms of crypsis, the darker the female, the less conspicuous she is.

Otherwise, the small, but statistically significant differences between males and females are rather questionable. The differences were found only within the ‘yellow’ population, while the sexes in the ‘lab’ population showed no distinction from each other. This may be interpreted in terms of small sample size or by the fact that many captive-bred lineages lose some of the ‘wild’ traits due to bottleneck effect, inbreeding, selective breeding etc. (Price 2002). The effect size is, however, relatively small. In the optimistic scenario, the effect size would be assessed as ‘medium’, but more probably would be even smaller (Ferguson 2009; Grice and Barrett 2014). Although males were often found at the upper extremes, while females at the lower extremes of the values, the percentage of non-overlapping data was 0-6%.

This may suggest that if any sex differences in coloration exist, they do not result from the sexual selection, but can be rather influenced physiologically, e.g. hormonally or by the temperature during incubation (Deeming et al. 1991). Existence of sexually dichromatic nocturnal animals is not much expectable due to the fact, that selection for conspicuous colors during the night makes little sense. Yet, geckos, having night color vision, would be potential candidates for such a phenomenon. For instance, geckos of the genus *Sphaerodactylus* often express a clearly distinct sexual dichromatism, although only a few species are believed to be nocturnal, e.g. *S. leucaster* or *S. roosevelti* (Gamble et al. 2015; Regalado 2015).

For future research, we suggest that manipulative behavioral experiments concerning UV vision in anti-predatory or social context should be carried out. The reaction of geckos’ predator (snakes, birds) on manipulated colors on neck and tail, especially in young geckos, would shed more light on this topic. We did not find any pronounced sexual dimorphism in colors that should be indicative for further testing of the role of overall color pattern in male-male competition or the importance of male coloration in mate choice in this species. However, in order to isolate the role of UV and particular colors or melanin-based pattern from other factors, like male residency and body size, it will require additional experiments with manipulated UV signals on males. We conclude that the current pattern of color change follows rather ontogenetic change in antipredation behavior of Leopard geckos. Our findings are more in concordance with antipredation hypothesis although the apparent UV signal on the tail of adult geckos may serve as a cue for both, interspecific as well as intraspecific communication at the same time.

Compliance with Ethical Standards

This work was supported by the Grant Agency of Charles University (No. 1636218/2018) and SVV project 260434/2019. Participation of P. F. was supported by the Charles University Research Centre program No. 204069.

The authors declare that they have no conflict of interest.

This study was performed in accordance with Czech law implementing all corresponding European Union regulations. Taking photos do not require application of anesthesia, surgical procedures or immobilization.
Human participants were not involved in this research.

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Table 1 T-test results for sex differences in pattern and luminance in the ‘yellow’ population.

		Mean – males	Mean – females	SD males	SD females	t-value	df	P*	Hedge's g
Mean luminance of white and grey patches on the tail (%)	White – luminance channel	35.36	30.92	8.9	7.14	3.13	142	**0.0021**	0.5749
	Grey – luminance channel	19.89	16.52	8.19	5.84	2.773	142	0.0063	0.5094
	White – UV channel	28.08	25.48	5.7	5.17	2.651	142	0.0089	0.487
	Grey - UV channel	16.23	13.88	5.77	4.5	2.608	142	0.0101	0.4791
Tail – luminance channel	Mean luminance (%)	14.48	12.26	3431.96	2352.83	2.99	148	**0.0033**	0.5335
	Total energy	10022.52	8824.72	2639.77	2038.83	2.995	148	**0.0032**	0.5344
	Proportion of area with >8% luminance	0.59	0.54	0.13	0.12	2.416	148	0.0169	0.431
Tail – UV channel	Mean luminance (%)	10.68	9.77	1870.14	1486.27	2.071	148	0.0401	0.3695
	Total energy	8108.19	7351.53	1853.18	1678.63	2.437	148	0.016	0.4349
	Proportion of area with >8% luminance	0.52	0.5	0.13	0.11	1.297	148	0.1968	0.2313
Back – luminance channel	Mean luminance (%)	16.06	13.15	3651.7	2355.53	3.801	148	**0.0002**	0.6782
	Total energy	8826.75	8083.62	2164.92	1740.24	2.212	148	0.0285	0.3946
	Proportion of area with >8% luminance	0.69	0.6	0.12	0.11	4.301	148	**<0.0001**	0.7674
Back – UV channel	Mean luminance (%)	6.23	5.84	1003.29	732.91	1.72	148	0.0876	0.3068
	Total energy	4080.54	3571.81	1444.98	1080.85	2.368	148	0.0192	0.4225
	Proportion of area with >8% luminance	0.31	0.29	0.14	0.11	1.215	148	0.2264	0.2168

* Significant values after Bonferroni correction (α = 0.005) are marked in boldface

Fig. 1 Comparison of photographs in visible and UV spectra. a 15 days old juvenile. b the same individual as a subadult, 300 days old. c and d. Two males from the 'yellow' population with high and low contrasting UV patterns.

Fig. 2 a Detail of an adult's tail with measured color. b The white crescent on the side of a juveniles head. c A pseudo-UV image of an adult gecko (without the limbs) with an output from Barvocuc software.

Fig. 3 The proportion of black, white+grey and yellow colors in males and females from the 'yellow' population. Grey columns stand for the sum of white and grey areas. Horizontal line – median, box – 25-75%, whiskers – non-outlier range, $*P < 0.001$.

Fig. 4 a Luminance of white (white boxes) and grey (grey boxes) patches on the tail of juveniles, subadults and adults. Plain boxes stand for luminance in visible spectrum, hatched boxes for UV spectrum. Horizontal line – median, box – 25-75%, whiskers – non-outlier range. b The proportion of black (black box), white+grey (grey box) and yellow (yellow box) colors in juveniles, subadults and adults. Grey columns stand for the sum of white and grey areas. Horizontal line – median, box – 25-75%, whiskers – non-outlier range.

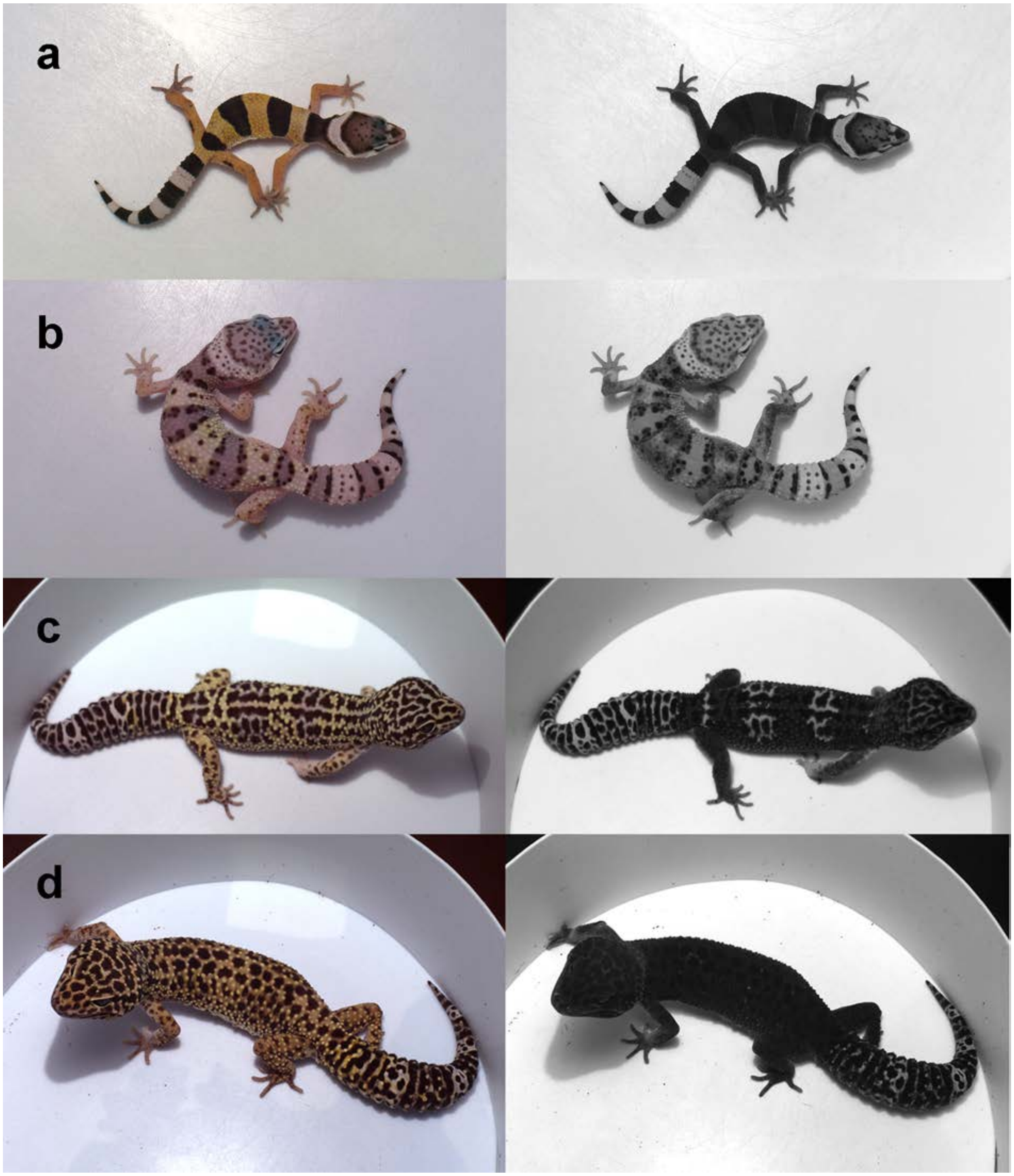

Figure 1

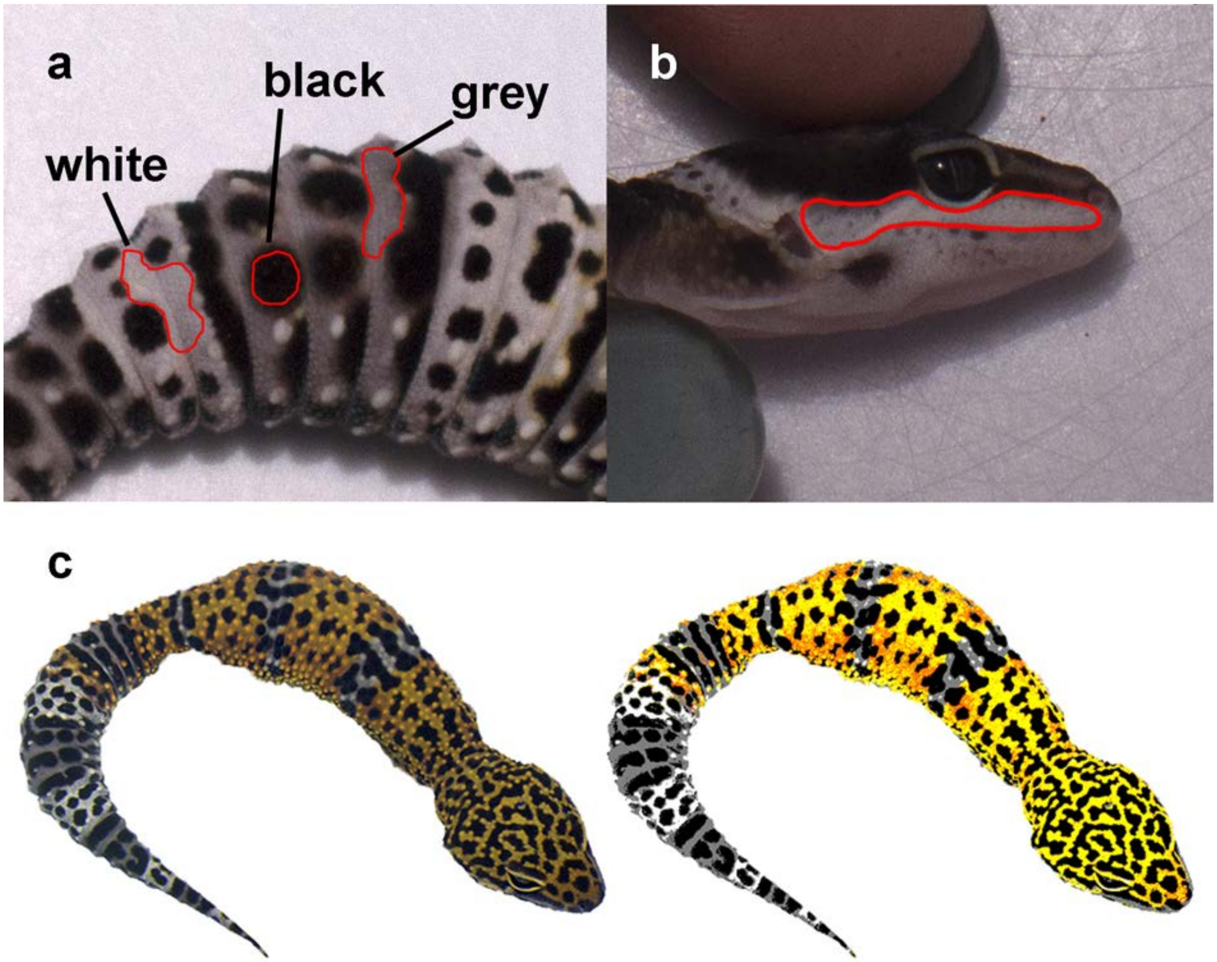

Figure 2

2

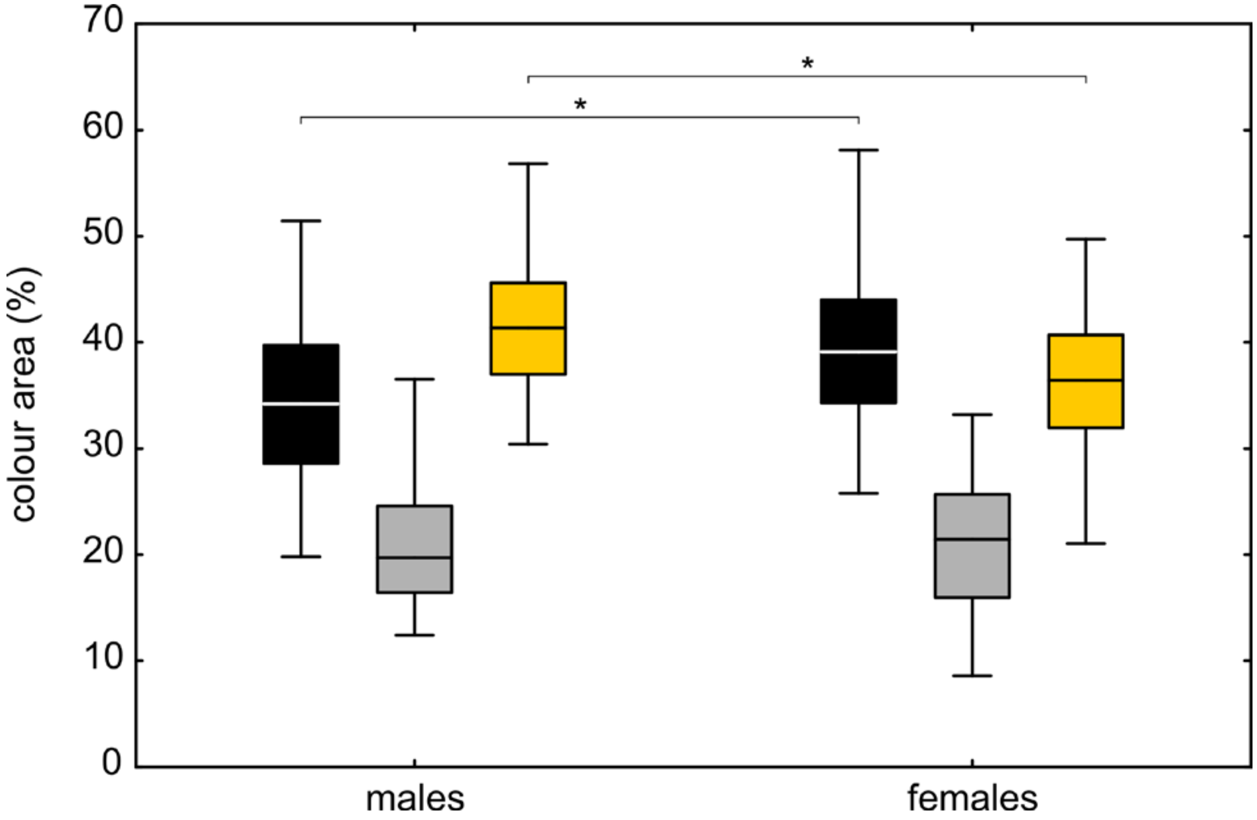

Figure 3

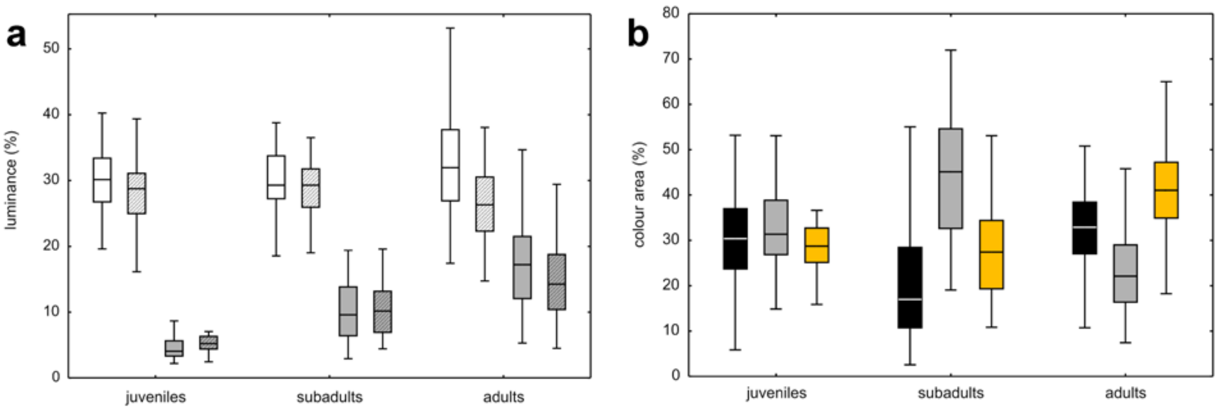
a
luminance (%)
0
10
20
30
40
50
juveniles
subadults
adults
b
colour area (%)
0
10
20
30
40
50
60
70
80
juveniles
subadults
adults

Figure 4

III

Snakes, ‘flags’ and contrasts: analysing conspicuousness of aposematic pattern through eye-tracking and visual modelling

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Submitted to Biological Journal of the Linnean Society

Snakes, ‘flags’ and contrasts: analysing conspicuousness of aposematic pattern through eye-tracking and visual modelling

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Running head: Aposematic colouration in an eye-tracking study

Keywords: aposematism, conspicuousness, eye-tracking, chromatic contrast, achromatic contrast, Serpentes, visual perception

Abstract

Conspicuous aposematic colouration is typically linked to high evolutionary cost, as it makes its bearers visible and attracts attention of potential predators. To cover for this disadvantage, the aposematic pattern should have a deterring effect to as many possible predators as possible. In this study, we examined the effectiveness of aposematic tri-coloured pattern of milk snakes on visual attention of human respondents (trichromatic vision) and we modelled the visual contrast as seen by other potential predators (di- and tetrachromats). For this purpose, we decomposed the aposematic pattern into nine coloured triads ('flags') and we measured eye movements of 34 Czech respondents using the eye-tracking camera. The results show that the most number of fixations as well as the longest total dwell time were pointed towards the colour boundaries with high chromatic contrast. Concurrently, results from the visual models show that the presence of the black-yellow contrast yields a high chromatic contrast for dichromats, while presence of red enhances vision of tri- and tetrachromats against green background. Thus, the aposematic pattern of milk snakes consists of a balanced mixture of hues and contrasts that makes the snakes conspicuous to a wide variety of predators.

Introduction

Warning colouration, especially among terrestrial animals, is often limited to the same palette, whether the species is an insect, amphibian or reptile (Edmunds, 1974; Darst et al., 2006, Ruxton et al., 2004). Various combinations of long wavelength colours – red, orange, yellow (Sillén-Tullberg 1985; Ritland, 1998; Exnerová at al., 2006b; Aronsson & Gamberale-Stille, 2008; Svádová et al., 2009) and sometimes white (Jones, 1932; Kettlewell, 1965; but see Stimson & Berman, 1990; Lyytinen et al., 1999; Exnerová et al., 2006a; Rönkä et al., 2018) together with black contribute to typical aposematic patterns (Poulton, 1890; Cott, 1940; Komárek, 2003; Exnerová et al., 2003). Several hypotheses have been proposed, explaining why these particular colours, and not others, carry the warning information (Ruxton, et al., 2004; Stevens & Ruxton, 2012). Long wavelengths seem to be most contrasting against green natural backgrounds (Aronsson & Gamberale-Stille, 2009) and unlike blue or white, they maintain more stable appearance under varying light conditions (Arenas et al., 2014). Aposematic animals may differ in hue and brightness—having chromatic and achromatic contrast to the background or to other objects (Olsson et al., 2018). Achromatic patterns are utilized in texture and edge discrimination and as such received a lot of attention (e.g., Osorio et al., 1999; Jones & Osorio, 2004). However, chromatic contrasts are believed to be more important in object recognition (Wichmann et al., 2002; Spence et al., 2006; Liebe et al., 2009). Together, these components are crucial for signal recognition (Fleishman & Persons, 2001; Prudic et al., 2006; White et al., 2017) and conspicuousness (Stobbe & Schaefer, 2008), which subsequently enhances avoidance learning and further generalizing to other aposematic patterns (chromatic contrast: Gittleman & Harvey 1980; Roper & Redstone 1987; Lindström et al., 1999; Exnerová et al. 2009; achromatic contrast: Prudic, et al., 2006). Certain species were also reported to have innate predisposition for awareness towards aposematic patterns (in birds: Smith, 1975); Caldwell & Rubinoff (1983), which seemed to differ from mere neophobia (Exnerová et al., 2006b).

It should be noted, however, that most of the studies dealt with birds as predators and insects (and to lesser extent snakes) as the prey (for general overview, see, e.g., (Ruxton *et al.*, 2004). Birds are mostly tetrachromats, yet, there are also di- and trichromatic mammals (Schaefer et al., 2004; Hegna et al., 2011; Kraemer et al., 2016) or tri- and tetrachromatic reptiles (Macedonia et al., 2009) and amphibians (Hatle & Salazar, 2001) to which the warning signals can be addressed as well. This brings us to a question how much universal the aposematic palette is. It makes sense to assume that the typical warning colouration is targeted as universally as possible, given its widespread presence in various taxa. Nonetheless, there may be partial, but biologically relevant differences in its perception based on the visual system itself.

In the present study, we aimed to explore the effect of aposematic colouration on trichromatic human vision, using eye-tracking technique and visual modelling. Eye-tracking has been widely used in marketing surveys (Maughan et al., 2007; Wedel & Pieters, 2008), exploration of the visual attention aimed to animal stimuli (Rinck, et al., 2005; Quinn, et al., 2009) and salience of emotional pictures (Nummenmaa et al., 2006) by recording eye saccades and fixations over particular areas of an image. In detection tasks, eye-tracking can provide specific information about gaze patterns and provide information about perceptual bias, which can expand our understanding of complex mechanisms of threat detection (LoBue et al., 2014). Patterns of visual attention or avoidance are firmly linked with autonomic nervous response and coping styles of cognitive risk evaluation (Aue et al., 2013).

Visual modelling is a different approach, when a mathematical approximation of photoreceptor responses to visual stimuli enables us to quantify colour and luminance contrasts perceived by particular species (Endler & Mielke, 2005; Osorio & Vorobyev, 2005).

We combined these two approaches in order to find out what eye movement patterns the aposematic stimulus elicits in humans, to what extent they can be explained by chromatic or achromatic contrasts and how the contrast perception differs in various visual systems. For this purpose, we designed our stimuli on the basis of the warning colouration of milk snakes (*Lampropeltis*, Colubridae), which we previously used as a model group for exploring human perception of snake beauty (Marešová et al., 2009) and dangerousness (Landová et al., 2012).

Milk snakes are Batesian mimics of some mildly venomous colubrids (e.g., *Pliocercus, Erythrolamprus*) and highly venomous elapids (*Micrurus*) (Greene & McDiarmid, 1981; Pfennig et al., 2001). Various kinds of predators, including other snakes, raptors, puffbirds (*Malacoptilus*), kiskadees (*Pitangus*), motmots (*Eumomota*), opossums, coatis and other carnivorous mammals have been reported to prey upon these snakes or attack their replicas (Smith, 1969, 1975, 1976; Brodie, 1993; Beckers et al., 1996; Buasso et al., 2006; Kikuchi & Pfennig, 2010). It was experimentally demonstrated that aposematic tricolour coral snake pattern and partly also bicolour striped pattern may provide efficient protection against predation in natural situations (Brodie, 1993; Brodie & Janzen, 1995; Hinman et al., 1997; Buasso et al., 2006).

However, reaction of primates to warning coral snake pattern is dependent on a different ability of colour vision (Lucas et al., 2003; Jacobs, 2008). Most New World monkeys living in sympatry with coral snakes and their mimics are allelic trichromats, i.e., trichromacy occurs only in heterozygous females, while the others are dichromats (for a review, see Jacobs et al., 1996; Jacobs, 2008; Surridge et al., 2003). In contrast, Howler monkeys of the genus *Alouatta* as well as all species of Old World monkeys and apes including human beings share fully developed routinely trichromatic colour vision (Dulai et al., 1999; Rowe, 2002) and thus, both sexes can perceive aposematic patterns similarly as other visually oriented predators, e.g., birds. However, humans have not encountered coral snakes or milk snakes during their evolutionary history, as those live in America, while human evolution took place within Africa and the Old World (Grine et al., 2009). Besides that, unlike the majority of mammals, humans share with birds the ability to discriminate red from green (Jacobs, 1993; Fernandez & Morris, 2007; Osorio & Vorobyev, 2008). The effect of red colour on human emotions (Young et al., 2013) and behaviour is extensively studied (Elliot et al., 2007; Elliot & Maier, 2014). This colour is believed to play a very important role in human (and primate) communication including signalling of sexual arousal and attractiveness (e.g., Roberts et al., 2010; Elliot et al., 2013), dominance and threat (Fetterman et al., 2011), and may facilitate either approach or avoidance behaviour depending on the context (Meier et al., 2012). Moreover, humans are able to recognize general aposematic patterns as had been demonstrated in studies modelling artificial evolution of aposematic forms (Sherratt & Beatty, 2003; Beatty et al., 2004; Bohlin et al., 2012).

For those reasons, we considered the milk snakes' warning colouration suitable for testing its potentially universal effect on visual attention (Marešová et al.. 2009), especially in relation to photoreceptor responses. In order to examine the effect of aposematic colouration per se, we avoided depicting the snake shape, which is known to often trigger emotional reaction in human brain (Almeida et al., 2015; Prokop et al., 2018) and attract prioritized attention (Lobue & Deloache, 2011), and we further decomposed the pattern to combinations of individual colours – red, black, and white/yellow.

Specifically, the aims of this study were to (1) examine the importance of the chromatic versus achromatic contrasts of tricoloured pattern for human visual attention, especially when red colour is present; (2) compare the results with contrasts modelled for visual systems of tetra- and dichromats, and (3) interpret the results in context of the effectiveness of milk snake warning colouration.

Material and methods

Eye-tracking

We created a set of 54 stimuli, each of them represented by a rectangular ‘flag’ (aspect ratio 1.77:1) with three identically wide vertical contrasting (“aposematic”) stripes. Each stripe had different colour (red, black, white, yellow). The particular shades were chosen by the following procedure. Five people were shown three live milk snakes *Lampropeltis triangulum* (newly *L. polyzona*; Ruane et al., 2014) under natural illumination and then they were asked to pick the closest red, black, white, and yellow shades from a PANTONE® Colour Formula Guide (17th edition, fourth printing, 1984-1985, Moonachie, New Jersey). CIE L*a*b* equivalents of the chosen shades, retrieved from Adobe Photoshop CS6 ‘PANTONE solid’ palettes, were used to calculate an average shade for each colour: red (L = 45, a = 55, b = 48), black (L = 15, a = 4, b = 2), white (L = 93, a = 0, b = 8), yellow (L = 92, a = -8, b = 86). As a control colour, which is absent from both the snake and its natural background, we added a blue stripe, equiluminant to the red (L = 45, a = 12, b = -60). Red, black, white, and blue stripes were used in all possible combinations, making up four series by six permutations of each colour triad. Three more series were added by duplicating ‘flags’ containing a white stripe and replacing it with yellow. The reason is that white and yellow represent the same type of ring in milk snakes, being part of a continuum. Finally, we added two series of greyscale versions of the ‘flags’ to have ‘luminance only’ stimuli (Fig. 1).

Thirty-four respondents (27 females, 7 males, average age 26.7 ± 7.2 years) attended the experiment. None of them reported colour-blindness. They were presented the stimuli on a calibrated 19-inch monitor (resolution 1280 x 1024 pixels, refresh rate 60 Hz), which was situated 70 cm from the respondent. The location of the respondent’s head was fixed using a chinrest. The stimuli were presented to the respondent in a random order and the eye movements were measured using the EyeLink1000 eye tracking device and an experimental setup designed using Experiment Builder (SR-Research). At the beginning of each presentation, the respondent answered four questions: their age, gender, whether they were right or left handed and their country of origin. The device was calibrated using the manufacturer's procedure when the respondent was asked to look at 9 points, successively appearing on the screen at nine different locations (centre of the screen, corners, centres of the edges) in a random order. The calibration was then validated using the same method of displaying the points and comparing the predicted eye position with the tracking data. The maximal allowed error was 1° of visual angle and average allowed error was 0.5°. If the error during validation was higher than allowed, the device was adjusted and calibration and validation was repeated. Once the validation was complete, the stimuli were presented for 5 seconds in a random order. Drift check was performed before each stimulus. The presentation longer than 30 stimuli had a break in the middle, where the respondent was allowed to rest his eyes and stretch. The presentation of stimuli was resumed after the respondent said that he/she was ready to continue. The subsequent presentation began by calibrating the device and validating the measurements using the method described above.

The data were extracted using DataViewer (SR-Research). The stimuli were divided in 12 areas of interest (4 for each colour), each interest area spanned the full length of the stimulus and one quarter of the width of the colour (Fig. 1b). For comparison of the contrasts, we used only the interest areas adjacent to a different colour (because retinal ganglion cells respond only to changes in light/colour and not to uniform patches; Snowden et al., 2012. This is probably also why the majority of fixations were on the edges.). To calculate the number of fixations (fixation count) and time spent looking at the contrast (dwell time), we added up the respective values from the two adjacent interest areas of different colours. Then we counted the proportion of the fixation count and dwell time on each contrast and averaged the numbers from the same contrasts from two mirror images (e.g., the black-red contrast from the first and last image from the first row in Figure 1a were averaged).

Visual modelling

Two data sets for visual modelling were obtained. Both were treated separately. 1) RAW photographs of the ‘flags’ screened on the eye-tracking monitor were taken with SamsungNX1000 and 35mm Novoflex Noflexar lens. The camera was set under the same illumination and at the same distance, from which the tested subjects viewed the monitor. The photographs were processed according to the methodology of Troscianko & Stevens (2015) with Multispectral Image Calibration and Analysis toolbox, a freely available plug-in for visual modelling (Troscianko & Stevens 2015), running on ImageJ platform (Schneider, Rasband, & Eliceiri, 2012). Photographs were calibrated against white screen (L = 100, a = 0, b = 0), i.e., the most luminant signal reached at the actual monitor settings. Functions for calculations of chromatic and achromatic contrasts, following the models of (Vorobyev & Osorio, 1998) and (Siddiqi et al., 2004), respectively, are integrated within the MICA toolbox, as well as receptor sensitivities of various species. We calculated the contrasts for visual systems of potential predators: dichromat (model ‘ferret’, cone abundance ratio SWS:LWS 1:14 (Calderone & Jacobs, 2003), trichromat (model ‘human’, SWS:MWS:LWS 1:16:32 (Vorobyev & Osorio, 1998), violet sensitive (VS) tetrachromat (model ‘peafowl’, UVS:SWS:MWS:LWS 1:1.9:2.2:2.1 (Hart, 2002), UV sensitive (UVS) tetrachromat (model ‘blue tit’, UVS:SWS:MWS:LWS 1:1.92:2.68:2.7 (Hart et al., 2000). Achromatic contrast calculations were based on the LWS receptor in the dichromat model (Osorio & Vorobyev, 2005), averaged MWS+LWS response in the ‘human’ model (Livingstone & Hubel, 1988;

Dacey, 2000) and double cones in the tetrachromats (Osorio & Vorobyev, 2005). Weber fraction was always set to 0.05.

2) Reflectance spectra were measured from eight adult milk snakes belonging to the following species or subspecies (the individuals were partially different from those used in creation of eye-tracking stimuli): *Lampropeltis triangulum campbelli*, *L. t. nelsoni*, *L. t. sinaloae*, *L. t. hondurensis*, *L. pyromelana woodini*, *L. ruthveni*, *L. getula*, *L. californiae*. According to the revised taxonomy of milk snakes, *Lampropeltis triangulum campbelli*, *L. t. nelsoni* and *L. t. sinaloae* are synonymized under *L. polyzona*, and *L. t. hondurensis* under *L. abnorma* (Ruane et al., 2014). Red, black and yellowish white (henceforth referred to as ‘cream’) rings were measured on each snake (Fig. 2), using OceanOptics spectrophotometer USB4000 and PX-2 Pulsed Xenon light source, calibrated against WS-1 white standard. The probe was held under 45° angle to avoid artefacts caused by the glossy surface of snake’s scales. All individuals were taken to measurements within a short time after their last skin shedding. For each colour, three measurements were taken (from the dorsal side of head, mid-body and tail, respectively) and then averaged. Avicol software (Gomez, 2006) was used for calculating chromatic and achromatic contrast with the same input parameters as in the previous case. We calculated contrasts of black, red and cream against each other as well as against various natural backgrounds. We used a set of spectra included in MICA toolbox (Troscianko & Stevens, 2015); leaves (n = 18), dead leaves (n = 45), grass (n = 20), twigs (n = 30), oak bark (n = 79).

Statistical analysis

To assess the differences between the dwell times and fixation counts for the contrasts, we used Linear Mixed-Effects model (R, command ‘lme’, package ‘nlme’) and a post-hoc Tukey test (R, command ‘lsmeans’, package ‘lsmeans’). In the LME model, we entered the identity of the contrast as fixed factor and the identity of the image and the respondent as random factors. We performed this analysis separately for each triad of colours (see fig 1a).

Results

Eye-tracking

We found no difference in the number of fixations to individual colours, whereas colour boundaries did draw attention. In most cases, there were no significant differences between particular colour pairs with three exceptions. In the black-red-white series, there was a significant effect of the contrast identity (F = 5.1582; df = 100; p = 0.0074). The black-red boundary drew more attention than black-white (p = 0.0168) or red-white (p = 0.0185). The differences were not significant in black-red-yellow series. In blue-red-white series (effect of contrast identity F= 7.9592; df = 100, p < 0.001), blue-red combination gained most attention compared to blue-white (p = 0.0045) or red-white (p = 0.0013). In black-blue-yellow series, there was a significant effect of contrast identity as well (F= 5.22422; df = 100; p = 0.007), however the Tukey test did not show any significant differences between the particular boundaries. In the analysis of the dwell time, we found a significant effect of the contrast identity for the black-red-white series (F = 6.2529; df = 100; p = 0.0028), for the black-blue-yellow series (F = 3.6548; df = 100; p = 0.0294), and the red-blue-white series (F = 5.3218; df = 100; p = 0.0064) (Fig. 3). All other combinations showed no significant differences in dwell time on different contrasts. In the black-red-white series, there was again no difference between dwell time on black-white versus red-white contrast (p = 0.6438), but there was a difference between the black-red versus red-white contrast (p = 0.0027) and black-red versus black-white contrast (p = 0.0358), with a preference of the black-red contrast.

In the black-blue-yellow series, there was a significant difference between black-blue and black-yellow boundary (p = 0.0448; black-yellow was preferred), but the blue-yellow boundary was different neither from the black-blue, nor from the black yellow-boundary ($p = 0.068$ and $p = 0.9823$ respectively).

In the red-blue-white series, there was no difference in dwell time between white-blue and white-red contrast ($p = 0.9432$), but the red-blue contrast was significantly more preferred than both blue-white ($p = 0.0253$) and red-white ($p = 0.0102$).

Visual modelling

In the ‘flags’ set, the achromatic contrasts exhibited similar pattern in all visual systems, with black-white and black-yellow at the upper extreme, equiluminant red-blue at the lower extreme and the remaining colour pair clustering in the middle (Fig. 4). Chromatic contrasts displayed similar pattern in human and bird visual systems, differing considerably from the dichromat ferret model. In tri- and tetrachromats, red-blue, followed by blue-yellow, had highest chromatic contrasts. In the human model, colour combinations involving black and colours with similar luminance (white and yellow, red and blue) clustered close to each other, with black-red being the most contrasting pair. The situation was alike in the birds, only with colour pairs involving blue being considerably more contrasting and separated from the pairs involving red (which occur naturally in aposematic snakes). In the ferret model, most contrasting colour pairs involved yellow. Unlike the other systems, black-yellow and red-yellow were more contrasting than black-red.

Contrasts calculated for the snakes set showed, in general, the same order as those from the ‘flags’ set (Fig. 4). Both black-red and red-cream combinations had high chromatic contrasts. Only in the ferret model, the chromatic contrasts were much more levelled with each other and their values were relatively low (colours within each pair were separated from each other by lesser number of discriminable shades) (Fig. 4). In the blue tit model, black-cream and red-cream values were shifted considerably towards high chromatic contrasts. In snake to background comparisons, some distinctions become more apparent (Fig. 5). In blue tit, unlike other visual models, the range of contrast values of cream colour against various backgrounds approaches the range of red-to-background values. Both bird and human models further show that the snake colours are clearly distinguishable from the background, while in ferret vision, the snake tends to be more cryptic (jnd < 1 in a number of cases). The post-hoc Tukey test revealed that the chromatic contrast of the black and red colours significantly differed in all models but ferret when seen against a green background and brown background – green background always had a higher chromatic contrast (see Table 1). In all four models, the achromatic contrasts within the snakes' pattern were on average higher than those between the snake and its background.

Discussion

The respondents’ attention to particular colour boundaries

Those colour pairs that were most preferred had also highest chromatic contrasts when compared to other boundaries within the same triad, i.e., black-red and blue-red. Their high salience was, however, also contingent on the third colour in the triad. Fixations on black-red, as well as blue-red dominated in the presence of white, but not yellow. This further emphasizes the importance of chromatic contrast because yellow colour yields chromatic contrast of similar strength as red, thus resulting in non-significant results when these two colours are compared within a single triad. The non-significant results of each of the achromatic colour pairs are also in agreement with this.

These results were confirmed by the analyses of both number of fixations and total dwell time. While number of fixations is usually linked to the search efficiency (Zelinsky, 1996; Zelinsky & Sheinberg, 1997), the total fixation time (dwell time) is correlated with preference (Glaholt et al., 2009). This suggests that both search efficiency and preference for certain colour boundaries are modulated by high chromatic contrast. The only difference was within the black-blue-yellow triad, in which the black-yellow boundary was preferred (in dwell time) when compared to black-blue and black-yellow; i.e., higher achromatic contrast was preferred. However, it may have also been an artefact, as the significance criterion was met marginally ($p = 0.045$). On the other hand, this case is consistent with findings of White et al. (2017), who tested detectability of black-blue-yellow frogs *Dendrobates tinctorius* (Cuvier, 1797) by human respondents and found both chromatic and achromatic contrasts to be mild predictors.

Within the black-red-white triad, black-red was attended to significantly longer and more often than the white-red contrast, although both boundaries have similar properties in terms of achromatic and chromatic contrasts. This observation may of course be an artefact, however, it can also be interesting to examine it with regards to the classic Berlin and Kay's (1969) theory of basic colour terms. These authors identified universal categorization of basic colours based on a world colour survey of 110 non-industrial languages (Kay et al., 2009). According to their theory, there are seven stages of lingual diversification of colour, with the first one (present in *all* of the languages) discriminating only the light-warm spectrum (incl. white, red and yellow) and the dark-cool spectrum (black, green, blue). Xiao et al. (2011) reported that partitioning of the categories into the warm-cool spectra is also biologically constrained. It is thus possible that the black-red boundary, which includes colours of the opposite category (dark-light), yielded a higher overall contrast within the eyes of our participants than the white-red colour boundary that lies within just the light/warm colour category (light-light) and thus attracting less attention. To resolve this, a more detailed study focused on the warm-cool spectra partitioning would be needed.

Perception of colour in di-, tri-, and tetrachromats

In tri- and tetrachromats, there is a noticeable overlap in the level of conspicuousness of black-red and black-yellow pairs as measured in snakes, having both chromatic and achromatic contrasts relatively high. The highest chromatic contrast in dichromat vision as modelled on the basis of 'flags' used in this study was black-yellow, followed by red-yellow and black-red. (The order was not quite similar for the live snakes, but this was caused by the absence of pure yellow in the snakes used in our study. Their cream colour was rather intermediate or closer to white). This may suggest that while red may be important for tetrachromatic predators as birds, it contributes little to the overall conspicuousness in the vision of carnivorous mammals, given the fact they lack red sensitive cones and sometimes may not even distinguish the shades of the snake from those of the background (Fig. 5). For dichromats, it may be the yellow rings that enhance the banded pattern primarily (see Fig. 3). It is also worth noting that yellow or white rings might substantially contribute to conspicuousness of the aposematic pattern in the eyes of the birds. Both black-cream and red-cream contrasts were shifted towards higher values in both bird models, especially in the blue tit. This may be due to the fact that the rings we see as white reflect no UV (Fig. 2; see also Kikuchi et al., 2014). Because the perception of white is conditioned by equal proportion of all wavelengths, the birds might perceive the colour of the rings as non-white, which would enhance the chromatic contrast. In the blue tit model, the contrast intensity of cream to background considerably overlaps with red to background contrast values. Indeed, both yellow-red rings and the coral snake pattern in general were shown to trigger alarm or avoidance in at least some species of birds (Smith, 1975, 1978). In sum, the typical coral snake triad black-red-yellow may be possibly advantageous in addressing both bird and mammalian predators.

Our models show that the achromatic contrasts within the snakes' pattern were on average higher than those between the snake and its background for di-, tri-, and tetrachromatic observers alike. It has been reported in humans that the achromatic visual system/magnocellular pathway performs the best when undergoing a shape discrimination task (Mullen & Beaudot, 2002; Macé et al., 2005), and early object recognition was shown to be facilitated rather by achromatic than chromatic contrasts even in some birds (Jones & Osorio, 2004). It is thus possible that the high achromatic contrast presented within the snake pattern functions simultaneously as disruptive (Honma et al., 2015), i.e., breaking the body outline (Endler, 2006), further expanding their effectiveness to various animal predators.

Importance of the red colour

High attention towards the red-blue boundary suggests that chromatic contrast is more important than achromatic. Both colours used in our test were equiluminant, but their combination was more attended and preferred over both red-white and blue-white pairs. In the black-red-blue triads, all three colour pairs, having high chromatic contrasts, received equal amounts of attention. Even though the difference was statistically insignificant, blue replacing red in the combination with black received slightly less attention. Red-black had also slightly higher chromatic contrast than blue-black, but the values were close so the difference could be found negligible. In literature, semantic role of red seems to be privileged over blue in a way (Elliot & Maier, 2014). Studies on colour-oriented attention in humans found that the red colour can capture and hold attention when watching emotionally salient stimuli (Kuniecki et al., 2015). Moreover, seeing red prior to a test can impair performance (Elliot et al. 2007). However, when considering preferences instead of attention, blue colour is usually most preferred when evaluating “beauty” or “aesthetic preferences” of various animals including birds (Frynta, et al., 2010; Lišková & Frynta, 2013; Lišková, et al., 2015), snakes (Landová et al., 2012; Ptáčková et al., 2017), and mammals (Rádlová, Landová & Frynta, 2018). Blue was also found to be preferred over red in gorillas and chimpanzees (Wells et al., 2008) and macaques (Humphrey, 1972; but in Skalníková, Frynta, Abramjan, Rokyta & Nekovářová, unpubl. data, the monkeys preferred red). In contrast, red usually either has no effect on human preferences of animals (Lišková et al., 2015) or is perceived negatively (Landová et al., 2018), with the exception of some snakes (Marešová et al., 2009; see also Rádlová et al. 2019, in which presence of red makes the snakes less disgusting in human eyes). In human respondents, the preferences for red colour is mainly present when evaluating potential mating partners (Elliot & Niesta, 2008; Pazda et al., 2012; but see Peperkoorn et al., 2016). In general, our results do not support strong, exclusive position of red, and rather emphasize the importance of chromatic contrast per se. Red is one of the most often-used colour in nature to increase conspicuousness and deter enemies, but it may be so because green grass and foliage is so common. In various habitats, different colour may serve as an effective signal of conspicuousness (Cazetta et al., 2009); even the blue colour, which is indeed utilized by some animals in an anti-predation strategy (Cooper Jr & Vitt, 1985; Mäthger et al., 2012).

Conspicuousness and evolution of coral snake mimetic complex

The above-mentioned combination of prey colours and patterns catch visual attention of potential predators because they are highly conspicuous. This attention is what makes conspicuousness costly. Mathematical models thus predict that only animals with good secondary defence can carry conspicuous warning signals (Sherratt & Beatty 2003, Endler & Mappes, 2004). However, the signal should be universal and affect multiple predators simultaneously to address at least half of the potential predators (Endler & Mappes, 2004). In case of Batesian mimicry, both model and its mimic pay the cost of conspicuousness. However, the mimic is not dangerous for its predators. Presence of mimetic species represent further cost for the model species, because predators might have learned preferences for conspicuous but palatable prey (mimetic) they encountered before. Dynamics of sympatric co-occurrence of the same colour pattern of the coral snakes and its mimics that copy the geographic variation is the classical example of this issue (Green & McDiarmind 1981). In their analysis, Rabosky et al. (2016) found evidence that coral snakes drive the distribution of harmless red-and-black banded snakes over the entire Western Hemisphere. When the coral snake is sympatric with its mimetic milk snake, being more conspicuous is advantageous for the model, while being more similar to the model is advantageous for the mimic. However, in the absence of models (coral snakes), the replicas of mimics are more frequently attacked by bird predators (Pfennig et al., 2001). In allopatric situation, or when models are rare, the mimetic milk snakes tend to be less conspicuous with more disruptive colour patterns (Pfennig et al. 2007; Pfennig & Mullen, 2010). Moreover, phylogenetical reconstruction of ancestral states gives the evidence of nineteen independent origins of mimicking coral snakes by various species of New world snakes as well as evidence of widespread evolutionary losses of mimetic colouration (Rabosky et al., 2016).

Our results show that visual attention is paid mostly to particular colour boundaries with high chromatic contrast (black-red, blue-red). The typical black-red pattern of live milk snakes and coral snakes is very similar to this (Marešová et al., 2009; Kikuchi et al., 2014). Moreover, visual modelling of alternative perceptions of colour by different species of potential predators shows that colour combinations forming the aposematic signals of milk snakes and coral snakes maximize its conspicuousness for tri- and tetra-chromats as well as for dichromats. Most importantly, the visual attention is attracted by both contrasting patches within the inner surface area (Aronsson & Gamberale-Stille, 2009) and its contrast with background (i.e., not just the colours per se; Gittleman & Harvey, 1980; but see Sillén-Tullberg, 1985). It is possible that this forms a part of a general phenomenon; however, more complex analysis of other aposematic complexes is needed.

The evolution of conspicuous pattern is linked to high costs (Endler & Mappes, 2004); however, the strongly contrasting pattern probably fully balances its disadvantage because it deters a wide variety of predators. Because the strongest attractor of attention is high chromatic contrast, it is possible that a different colour combination such as blue-yellow might be equally effective. For example, aposematic complex including the blue colour may work well in the species of dart frog *Dendrobates tinctorius* (Wollenberg et al., 2008; Noonan & Comeault 2008), but within snakes, blue colour is rare.

Ethical note

This study was carried out in accordance with the recommendations of Institutional Review Board (IRB), Faculty of Sciences, Charles University, approval n. 2013/7, and approval of the Ethical Committee of the National Institute of Mental Health n. 55/16, with the written informed consent from all subjects in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board (IRB).

Acknowledgements

We would like to thank to Tomáš Grim and Daniel Hanley for lending us the spectrophotometer, Anna Bauerová for assistance during spectrophotometric measurments of the snakes and Kristýna Sedláčková for additional assistance during the implementation of this project.

Funding

This study is a result of the research funded by the GAČR n. 17-15991S. Personal costs of SR and BŽ were covered by the project Nr. LO1611, with a financial support from the MEYS under the NPU I program.

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Figure captions

Figure 1. A) Set of 54 stimuli used in the eye-tracking experiment. B) Demarcation of interest areas. Sum of bordering areas *d* and *a* was used as an interest area for counting fixations to the colour border.

Figure 2. Reflectance spectra of black (black lines), red (red lines) and cream (yellow lines) stripes from live milk snakes.

Figure 3. Dwell times for colour triads in the series of ‘flags’, where significant differences were detected. Middle line = median, box = 25-75%, whiskers = non-outlier range, dots = outliers, * $p < 0.05$, ** $p < 0.01$.

Figure 4. Chromatic and achromatic contrasts of individual colour pairs (‘flags’ and snakes data sets) calculated for four visual systems (dichromat – ferret, trichromat – human, VS tetrachromat – peafowl and UVS tetrachromat – blue tit). Stars stands for average values. Please note different x scales in the ‘flags’ set.

Figure 5. Contrasts of black, red and cream against each other and against natural backgrounds, plotted for four visual systems. Zero equals to just noticeable difference (jnd) = 1, i.e. to the point below which two shades are not distinguishable.

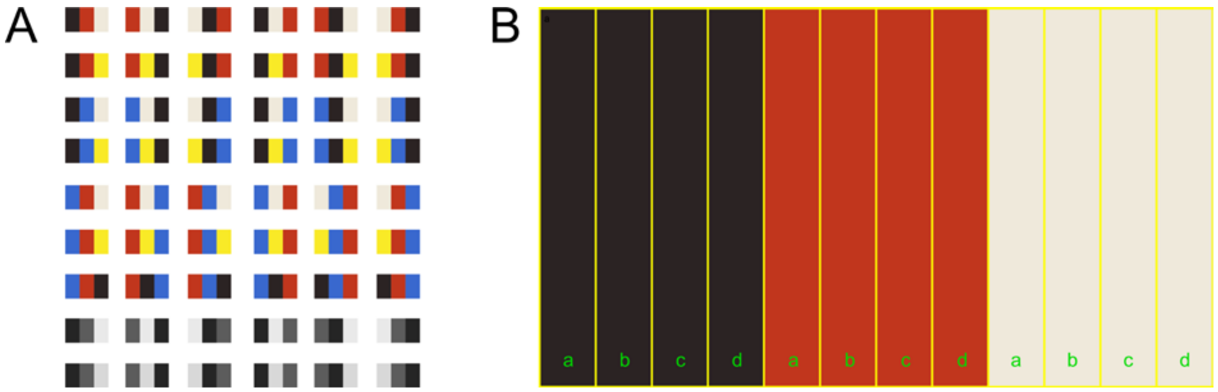
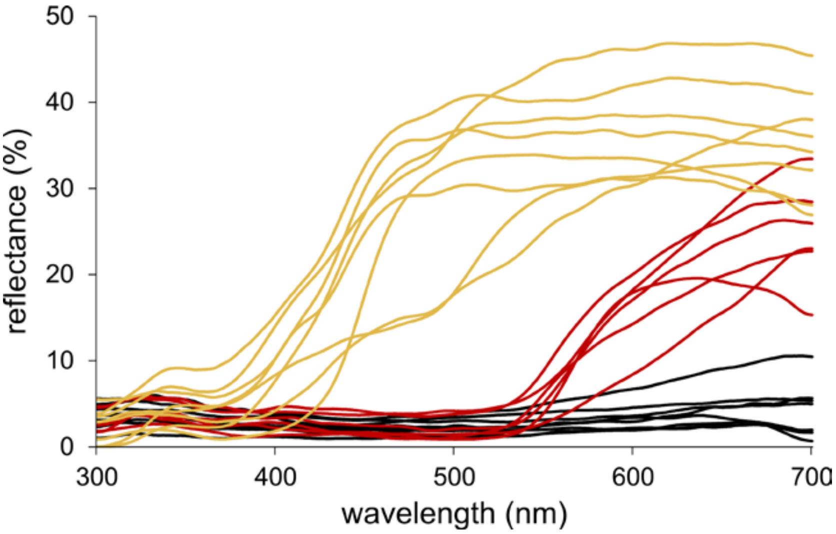


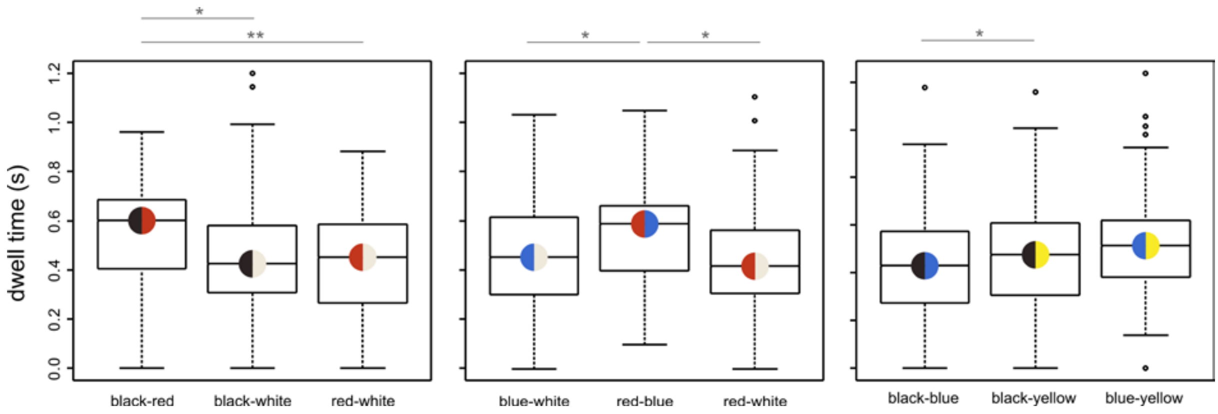
Figure 1.

826



827

828 Figure 2.


*
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dwell time (s)
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0.8
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1.2
black-red
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blue-white
red-blue
red-white
black-blue
black-yellow
blue-yellow

Figure 3.

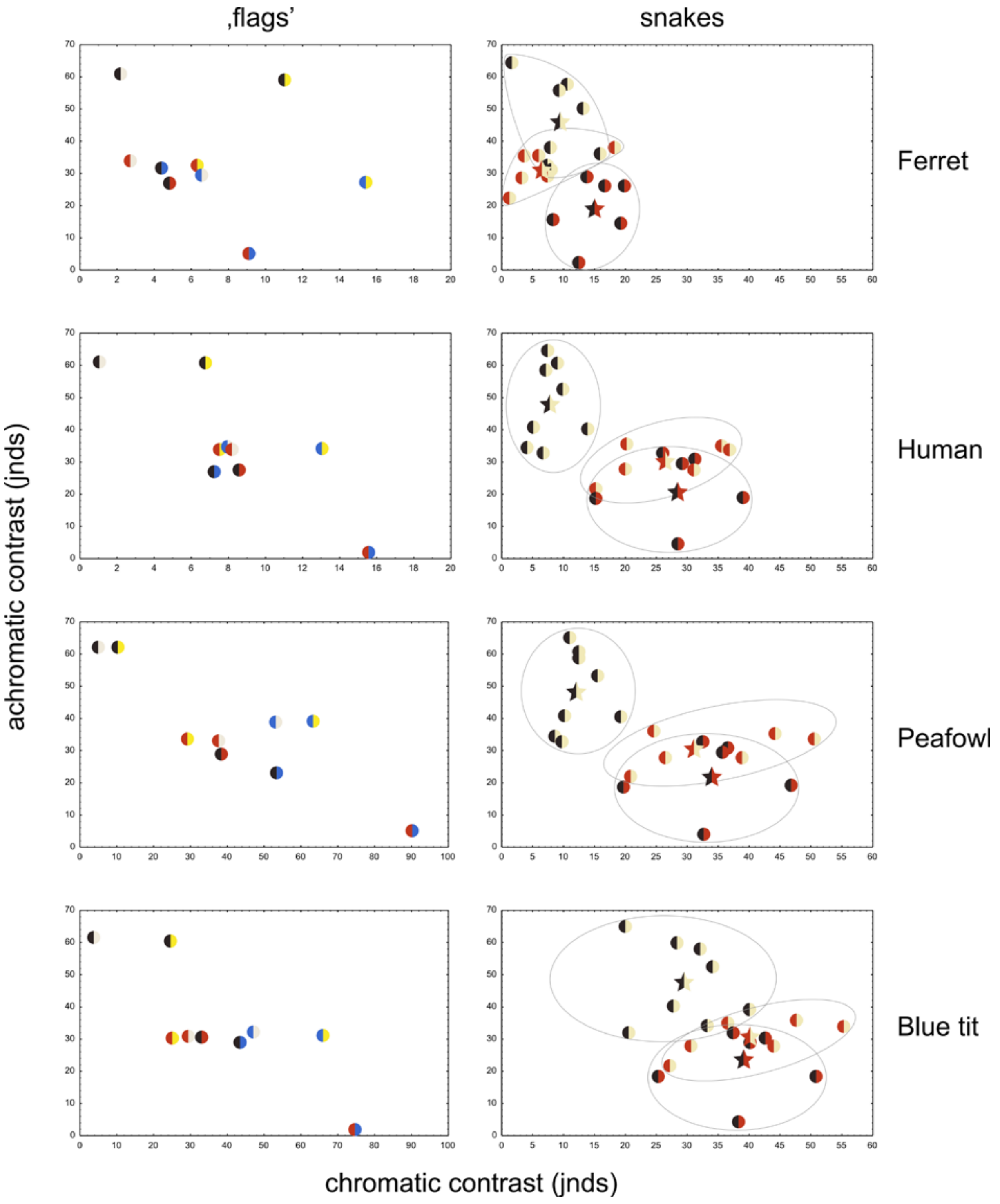
‚flags'
snakes
Ferret
Human
Peafowl
Blue tit
achromatic contrast (jnds)
chromatic contrast (jnds)
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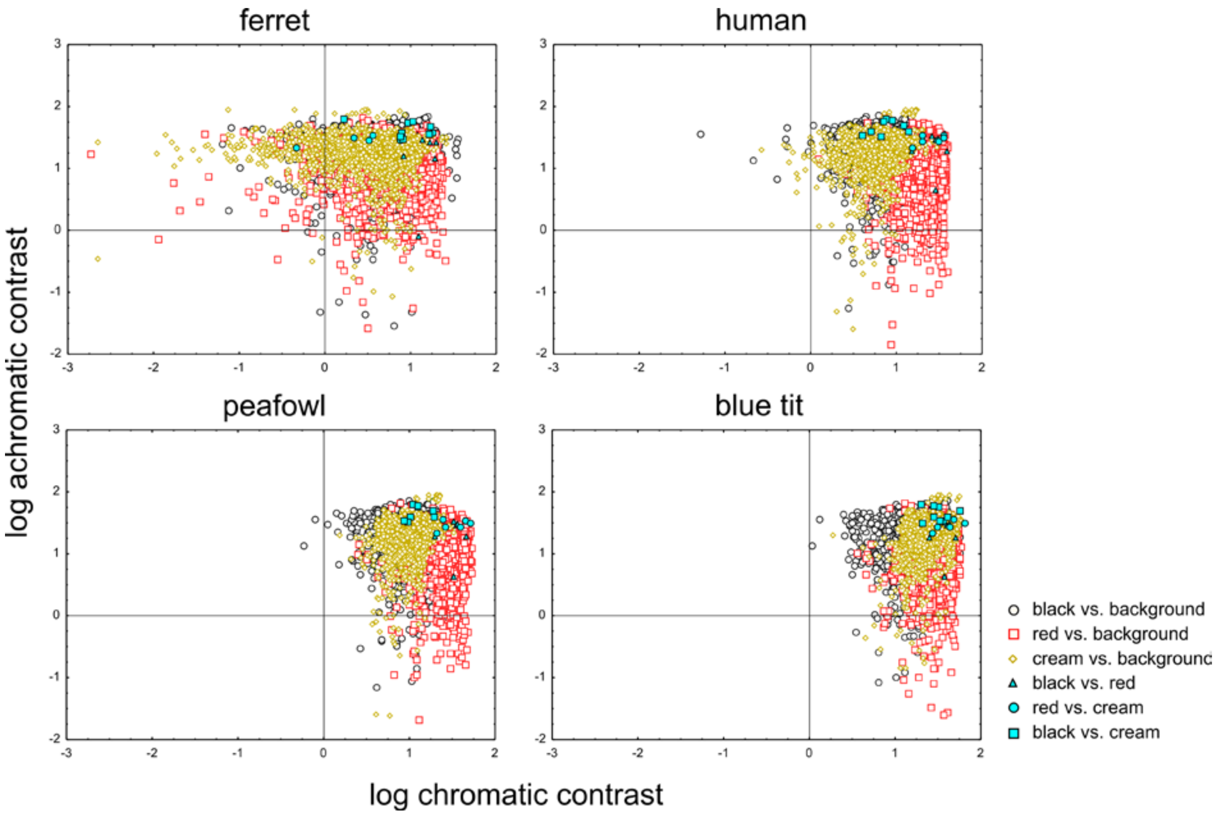
Figure 4.



836

837 Figure 5.

838

Table 1. Comparison of the green and brown backgrounds. The table shows P values of the Tukey test examining whether the given colour significantly differs when seen on a green background and when seen on a brown background. With the exception of ferret, chromatic contrast of the red colour always significantly differs when seen on a green background as compared to a brown background (green background has a higher chromatic contrast). Achromatic contrast does not play an important role.

a) Chromatic contrast

Colour	ferret	human	bluetit	peafowl
Black	<0.0001	<0.0001	<0.0001	<0.0001
Red	0.4824	<0.0001	<0.0001	<0.0001
Cream	0.4321	0.1809	<0.0001	<0.0001

b) Achromatic contrast

Colour	ferret	human	bluetit	peafowl
Black	0.3846	0.2226	0.8497	0.2119
Red	0.2741	0.0225	0.2351	0.0200
Cream	0.3999	0.1698	0.8997	0.2197

IV

Comparing developmental stability in unisexual and bisexual rock lizards of the genus *Darevskia*

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Evolution & Development: 1–*13*

Received: 9 July 2018 | Revised: 14 February 2019 | Accepted: 27 February 2019

DOI: 10.1111/ede.12286

RESEARCH

WILEY

Comparing developmental stability in unisexual and bisexual rock lizards of the genus *Darevskia*

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Funding information

Czech Science Foundation, Grant/Award Number: 18-15020S; Charles University Research Centre program, Grant/Award Number: 204069

Abstract

Parthenogenetic species are usually considered to be short-lived due to the accumulation of adverse mutations, lack of genetic variability, and inability to adapt to changing environment. If so, one may expect that the phenotype of clonal organisms may reflect such genetic and/or environmental stress. To test this hypothesis, we compared the developmental stability of bisexual and parthenogenetic lizards of the genus *Darevskia*. We assessed asymmetries in three meristic traits: ventral, preanal, and supratemporal scales. Our results suggest that the amount of ventral and preanal asymmetries is significantly higher in clones compared with their maternal, but not paternal, progenitor species. However, it is questionable, whether this is a consequence of clonality, as it may be considered a mild form of outbreeding depression as well. Moreover, most ventral asymmetries were found in the bisexual species *Darevskia valentini*. We suggest that greater differences in asymmetry levels among bisexuals may be, for instance, a consequence of the population size: the smaller the population, the higher the inbreeding and the developmental instability. On the basis of the traits examined in this study, the parthenogens do not seem to be of significantly poorer quality.

KEYWORDS

developmental stability, fluctuating asymmetry, lizards, morphology, parthenogenesis

1 | INTRODUCTION

Sexual reproduction is the predominant reproductive mode in complex organisms, especially vertebrates, and is considered to be one of the major drivers of evolution. Maintaining the genetic variability through sex and recombination is crucial for the ability to adapt, whether by coping with pathogens and parasites, or by avoiding the accumulation of harmful mutations, as has been suggested by several hypotheses (e.g., Müller's ratchet—Müller, 1964, Red Queen—Van Valen, 1973). Therefore, the occurrence of various unisexual species of fish, amphibians, or reptiles drew attention of evolutionary biologists. It was hypothesized that the side effects of clonal reproduction (the lack of genetic diversity together with accumulation of adverse mutations) could lead to an early extinction of a unisexual lineage, which is supported by the evidence that almost all of the recent parthenogens are of very young age (Dessauer & Cole, 1989; Freitas et al., 2016; Parker & Selander, 1976; Schmitz, Vences, Weitkus, Ziegler, & Böhme, 2001). Nevertheless, this concept has been questioned by the "neutral drift" hypothesis, which provides an alternative explanation for the young age of clones without the use of genetic decay (e.g., Janko, 2014; Janko, Drozd, & Eisner, 2011).

As for the poorer quality of clones, the empirical evidence has been rather inconsistent and the situation is further complicated by the fact that parthenogens often arise from crossbreeding. Several studies have shown the unisexuals to be more infested with parasites than their bisexual relatives (Bruvo, Schulenburg, Storhas, & Michiels, 2007; Kearney & Shine, 2004a; Lively, Craddock, & Vrijenhoek, 1990; Moritz, Mccallum, Donnellan & Roberts, 1991), to have lower survivorship (Johnson, 2000), be outcompeted in their habitats by the bisexuals (Dame & Petren, 2006), or to have somewhat worse performance (Cullum, 1997). Other studies, however, documented the opposite (Danielyan, Arakelyan, & Stepanyan, 2008; Tarkhnishvili, Gavashelishvili, Avaliani, Murtskhvaladze, & Mumladze, 2010). Besides this, most of the obligatory parthenogens—and lizards in particular—are proven to be of a hybrid origin (Dawley & Bogart, 1989; Grismer et al., 2014; Kearney, Fujita, & Ridenour, 2009; Simon, Delmotte, Rispe, & Crease, 2003; Uzzell & Darevsky, 1975). They often arise from crossbreeding of species, which are phylogenetically relatively distant (Jančúchová-Lásková, Landová, & Frynta, 2015). Although the hybridity may be advantageous in terms of high heterozygosity, it may also lead to the so-called outbreeding depression, which results from the disruption of coadapted gene complexes and leads to developmental instabilities (Clarke, 1993; Møller & Swaddle, 1997; Vøllestad, Hindar, & Møller, 1999).

Despite some controversies, fluctuating asymmetry has been widely used as a practical approximation of the ontogenetic stability. A number of studies found a connection between the level of stress (genetic or environmental) and deviation from the ideal bilateral symmetry (Lazić, Kaliontzopoulou, Carretero, & Crnobrnja-Isailović, 2013; Leary & Allendorf, 1989; Møller & Swaddle, 1997; Palmer & Strobeck, 1992; Parsons, 1992; Polak, Kroeger, Cartwright, & Ponce deLeon, 2004). In reptiles, temperature and/or population size were shown to be factors and has been largely discussed in the literature (Arnold & Peterson, 2002; Băncilă, Van Gelder, Rotteveel, Loman, & Arntzen, 2010; Lens et al., 2000; Löwenborg, Shine, & Hagman, 2011; Qualls & Andrews, 1999). As for hybridity, its effect on the developmental stability differs from case to case, although increased instability appears to be more frequent in interspecific hybrids rather than in crosses between subspecies, populations, or races (reviewed in Alibert, Auffray & Polak, 2003; Leamy & Klingenberg, 2005; Vøllestad et al., 1999). Therefore, there are reasons to expect that parthenogenetic hybrids may be less resistant to various kinds of stress than their bisexual relatives, which would be manifested on their overall body condition.

In our study, we focused on parthenogenetic and bisexual species of Caucasian rock lizards of the genus *Darevskia* and compared their developmental stability. Within the genus, seven obligatory parthenogenetic lineages arose independently from interspecific hybridization of four bisexual species; two of them being maternal ancestors and two of them being paternal ones (maternal species are related to each other more closely than to either of the paternal species and vice versa; Moritz et al., 1992; Murphy, Fu, MacCulloch, Darevsky, & Kupriyanova, 2000). Thus, we can compare different parthenogenetic lineages, all belonging to the same complex, but each of them having a unique combination of parental genomes. Recent findings even suggest that in some cases more than two parental species may have been involved (Tarkhnishvili, Murtskhvaladze, & Anderson, 2017). There is evidence that genetic introgression and backcrossing took place among various species of *Darevskia* lizards, so one should keep in mind that the genetic background of unisexual hybrids might be more complex (Darevsky, 1967; Freitas et al., 2016; Tarkhnishvili, Murtskhvaladze, & Gavashelishvili, 2013). All parthenogenetic *Darevskia* are diploid ($2n = 38$), which makes the overall assessment of their characters clearer as there are no triploid lineages, known to occur in other unisexual taxa (Danielyan et al., 2008; Darevsky, 1966; Schön, Van Dijk, & Martens, 2009). Thus, the "genome dosage" effect, which would complicate comparisons of bisexuals and unisexuals, may be ruled out in our case (Kearney & Shine, 2004a, 2004b).

In this paper, we test the hypothesis that the parthenogens should be more affected by developmental instabilities. We incline to the concept proposed by Shine, Langkilde, Wall, and Mason (2005), later used by Löwenborg et al. (2011), and define asymmetry not as a deviation from the zero population mean, but as an ontogenetic defect. For this purpose, we concentrated on aberrations in lizards' scalation, for example, missing or supernumerary scales. We assessed the rate of scalation asymmetries in *Darevskia* lizards, in four parthenogenetic lineages (*Darevskia armeniaca*, *Darevskia dahli*, *Darevskia rostombekovi*, and *Darevskia unisexualis*) and the corresponding maternal (*Darevskia mixta* and *Darevskia raddei*) and paternal (*Darevskia portschinskii* and *Darevskia valentini*) bisexual species.

2 | MATERIAL AND METHODS

The material used in this study comprises 587 individuals belonging to four parental bisexual and four unisexual species. 453 specimens (77%) were ethanol-fixed individuals from the herpetological collections of the Zoological Museum of the Moscow State University. Additional 134 individuals were captured in Armenia during July 2010 and 2012. After being measured, photographed, and scanned, lizards were released back into the wild. We examined 269 bisexual (132 males and 137 females) and 318 parthenogenetic individuals. Parthenogenetic species: *D. armeniaca* ($n = 137$), *D. dahli* ($n = 78$), *D. rostombekovi* ($n = 31$), and *D. unisexualis* ($n = 72$). Bisexual species: *D. portschinskii* ($n = 76$, 34 males, 42 females), *D. valentini* ($n = 38$, 21 males, 17 females), *D. mixta* ($n = 57$, 28 males, 29 females), *D. raddei* ($n = 98$, 49 males, 49 females). Two subspecies, *D. raddei raddei* and *D. raddei nairensis,* were treated together as *D. raddei*. They are considered as separate species by some taxonomists, however, their monophyly has been refuted by the molecular evidence (Freitas et al., 2016; Fu, Murphy, & Darevsky, 2000; Moritz et al., 1992). The morphological traits examined in our study did not significantly differ between the two (Darevsky, 1967; Petrosyan & Arakelyan, 2013). The complete list including localities and catalogue numbers is given in Appendix 1.

Each lizard was photographed with a digital camera (preserved specimens) or scanned (living individuals) using CanoScan LiDE 100 at resolution 600 dpi from both ventral and dorsal sides. Separate close-ups of the head were photographed from the dorsal, ventral, and lateral sides. In addition, we measured the snout-vent length (SVL) and head length (HL) using a digital caliper. Due to the condition of some specimens and/or technical difficulties, some traits could not be accessed, therefore the total N for preanal and supratemporal asymmetries is lower than for ventral asymmetries (bisexuals: $N = 234$, parthenogens: $N = 297$).

Sex was determined on the basis of femoral pores and hemipenes (where present at dissected museum individuals). In ambiguous cases, we also took into consideration the count of transversal ventral rows, mating period jaw marks on lizards' bellies and SVL:HL ratio, which are all sex-specific traits (Darevsky, 1967). Temperature is known to affect the incubation and subsequently the developmental stability in reptiles (Braña & Ji, 2000; Kearney & Shine, 2004a; Löwenborg et al., 2011; Qualls & Andrews, 1999). Therefore, we also recorded the altitude as a proxy of average local temperature using a GPS device in the field. For the museum specimens, the altitude was estimated according to the localities given in museum records and topographic Google Maps.

2.1 | Asymmetry evaluation

Digital images were used for examining the scalation (Figure 1). We evaluated three meristic characters: ventral, supratemporal, and preanal scales. Ventral scales are a highly repetitive element, arranged in 20–31 transversal rows, typically with six shields in a row. Deviations from the basic pattern can be clearly diagnosed. Most common anomalies (98%) are unilateral extra rows, while the rest are interrupted rows or merged neighboring scales (Figure 1a–c). All these cases were treated as a "ventral asymmetry", which was defined as an anomaly at a particular transversal row. The number of transversal ventral rows was counted (including unilateral extra rows) and the sum, order, and laterality of asymmetries recorded.

Preanals constitute an arc around the anal shield and have two possible configurations—odd or even—with 1–4 scales on each side from the central shield or a pair of shields, respectively. In 2% of cases, an intermediate pattern was present; it was not clear whether there were two central shields or just one. We treated all patterns according to the axis of body symmetry and counted the shields on both sides of the

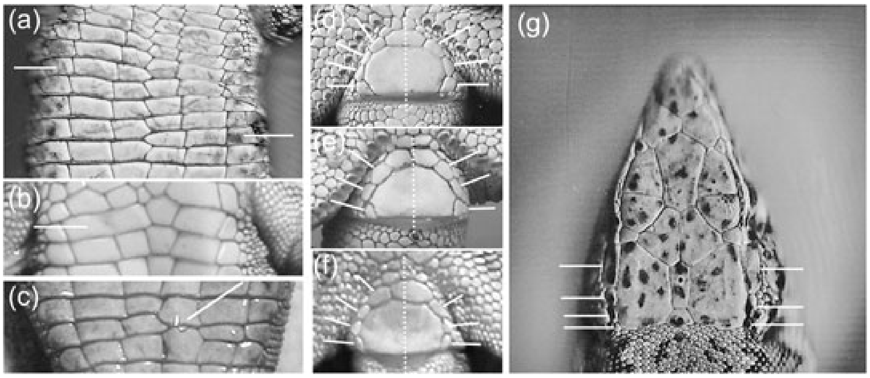

FIGURE 1 Examples of scale asymmetries. Ventrals: (a) unilateral extra rows (*Darevskia valentini*), (b) merged neighbor scales (*Darevskia rostombekovi*), (c) missing scale (Darevskia *dahli*). Preanals: (d) central shield pattern (*Darevskia armeniaca*), (e) two central shields pattern (*Darevskia raddei*), (f) intermediate pattern (*Darevskia unisexualis*). (g) Supratemporals (*Darevskia armeniaca*). Segment lines point to anomalies (a–c) or shields counted (d–g). Dotted line marks the axis of symmetry of the body

axis, excluding the shield the axis ran through or the two shields the axis ran in between (Figure 1d–f). A non-zero difference between the sides was defined as an asymmetry.

Supratemporals are made up of 2–7 scales on each side of the head between temporal and parietal shields. Their number was counted for the left and right sides and asymmetry was defined as a non-zero difference between the sides (Figure 1g). A difference bigger than 1 was in less than 1% in supratemporals and none in preanals, therefore only laterality and presence/absence of asymmetry was recorded for these traits. The data were checked for the criteria presented by Swain (1987), who argues for a correction in certain cases, when the meristic characters on both sides differ just a little (no more than 1–2). No correction was needed.

2.2 | Statistical analysis

We used R 3.5.0 software (nlme and geepack packages) for the calculations (R Core Team, 2013). The counts of ventral rows were compared between females of parthenogenetic and bisexual species to make sure the meristic background was the same for both groups. The Generalized Least Squares (GLS) method was applied with "species" as a random factor, treated with the "compound symmetry structure" and the output was calculated with the analysis of variance (ANOVA).

At first, we tested the bisexual species. We used generalized linear models (GLM) with species, sex, species × sex interaction, altitude, and SVL as possible factors influencing: (a) the amount of ventral scale anomalies per individual (VAA) and (b) the frequency of asymmetrical individuals per species (ventrals [VAI], preanals [PA], supratemporals [ST]). We were gradually excluding the least significant factors according to Akaike's Information Criterion (AIC), until we reached the most parsimonious model, which included only species. Then we ran the simplified model, replacing the "species" factor by "parental group" (maternal/paternal) and compared the two models with the "ANOVA" command. Parthenogens were analogously tested with the species, altitude, and SVL as factors.

Next, the simplified model was applied to the whole data set including both bisexuals and parthenogens. The result was checked over by the Generalized Estimating Equations (GEE) with "exchangeable correlation structure," "reproduction mode" as a factor and the "species" as a random factor to avoid the pseudoreplications due to the within-species similarity.

Laterality of asymmetries was examined with a two-tailed sign test.

3 | RESULTS

Comparison of the number of ventral rows between parthenogenetic and bisexual females by GLS revealed no significant difference between the two groups (mean ± standard error [*SE*] count of ventral rows for bisexual females: 27.42 ± 0.16, for parthenogens: 27.61 ± 0.06; $F = 0.062$, $P = 0.803$). In total, parthenogens had slightly higher, yet statistically insignificant mean ± *SE* number of ventral asymmetries per individual than bisexuals (0.80 ± 0.06 vs. 0.68 ± 0.06; $F = 2.26$, $P = 0.13$). For detected asymmetry values see Figure 2. Overview of asymmetry counts is given in Appendix 2.

In bisexual species, GLMs revealed significant effects of species on both ventral (VAA: $P = 0.003$; VAI: $P = 0.009$) and preanal ($P = 0.042$), but not supratemporal asymmetries ($P = 0.100$; Figure 2). In contrast, neither model confirmed the putative link between the "sex" and frequency of asymmetries in ventral, preanal, or supratemporal scales (VAA: $P = 0.936$; VAI: $P = 0.891$, PA: $P = 0.604$, ST: $P = 0.923$). In parthenogenetic species, GLMs revealed no effect of species on asymmetries in neither case, though the insignificance was liminal for supratemporals (VAA: $P = 0.216$; VAI: $P = 0.112$; PA: $P = 0.352$; ST: $P = 0.059$). This allowed us to pool the parthenogenetic species into a single "parthenogenetic hybrid" category for further analyses. For the effects of the individual factors, see Table 1.

ANOVA comparisons of full and reduced models confirmed that the posterior reduction of the original models had no effect on the results (VAA: $P = 0.304$; VAI: $P = 0.599$, PA: $P = 0.270$), with the exception of supratemporal asymmetries, where the "species" factor could not be reduced to the "parental group" factor ($P = 0.048$). Thus, we pooled the species belonging into each parental group together and performed alternative models on the whole data set, with the "three group" factor (maternal/paternal/parthenogenetic hybrid). In the case of supratemporals, a "five group" factor was used (*mixta*, *raddei*, *portschinskii*, *valentini*, and parthenogenetic hybrid) and a GLM model applied.

In the case of ventral and preanal asymmetries, GEEs revealed that the parthenogenetic hybrids showed no significant difference from the paternal group, but differed markedly from the maternal group (VAA: $df = 2$, $\chi^2 = 27.48$, $P < 0.001$; VAI: $df = 2$, $\chi^2 = 22.38$, $P < 0.001$; PA: $df = 2$, $\chi^2 = 85.2$; $P < 0.001$). Only the frequency of individuals with supratemporal asymmetries expressed no systematic pattern according to the GLM model ($df = 4$, deviance = 6.467, $P = 0.176$).

The sign test revealed a significant directional asymmetry in ventral scale anomalies, which prevailed on the left side in bisexual species (left 67.6%, $Z = 3.606$,

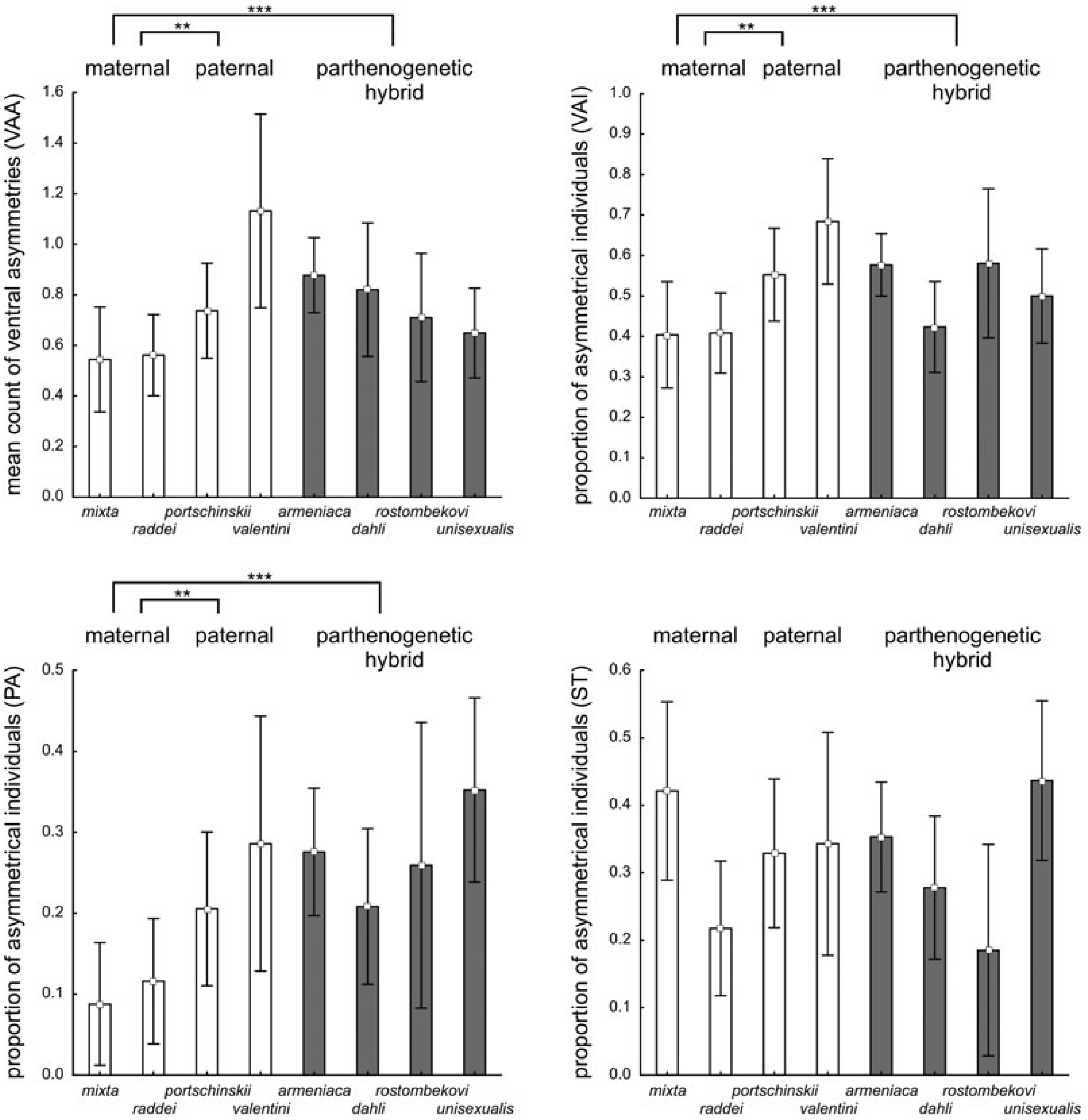

FIGURE 2 Values for ventral (VAA, VAI), preanal (PA) and supratemporal (ST) asymmetries. White columns: bisexual species. Gray columns: parthenogenetic species. Whiskers denote 95% confidence interval. Significance: $^{**}P < 0.01$, $^{***}P < 0.001$

$P < 0.001$), while neither side dominated in parthenogens (left 56.9%, $Z = 1.538$, $P = 0.124$). Most anomalies occurred on the chest. Thus, when analyzed separately, the results were comparable for the first five rows (bisexuals: left 69.3%, $Z = 3.233$, $P < 0.001$; parthenogens: left 59.2%, $Z = 1.717$, $P = 0.086$), while the trend was statistically insignificant for the rest of the belly (bisexuals: left 64%, $Z = 1.789$, $P = 0.074$; parthenogens: left 48.3%, $Z = 0.131$, $P = 0.896$). For the total distribution of ventral asymmetries, see Figure 3. Neither preanal nor supratemporal asymmetries were side-biased (PA, bisexuals: left 44.83%, $Z = 0.370$, $P = 0.710$; parthenogens: left 44.07%, $Z = 0.780$, $P = 0.435$; ST, bisexuals: left 44%, $Z = 0.924$, $P = 0.356$; parthenogens: left 54%, $Z = 0.700$, $P = 0.484$).

4 | DISCUSSION

Our data show that the amount of developmental stability based on the three meristic traits is comparable between parthenogens and their bisexual progenitors in the lizards of the genus *Darevskia*. Some partial differences were

TABLE 1 ANOVA tables for the effect of species and/or sex on incidence of scale asymmetries in bisexual and parthenogenetic species of the Caucasian rock lizards of the genus *Darevskia*

		VAA				VAI			
Model	**Factors**	***df***	**Dev.**	**Res.dev.**	***P***	***df***	**Dev.**	**Res.dev.**	***P***
Full model (GLM) for bisexuals	Species	3	13.638	265	**0.003**	3	11.400	361	**0.010**
	Sex	1	0.006	264	0.936	1	0.020	361	0.892
	Altitude	1	1.045	262	0.307	1	0.490	361	0.482
	SVL	1	2.234	261	0.135	1	2.300	359	0.129
	Species × Sex	3	1.913	258	0.591	3	4.320	354	0.229
Reduced model (GLM) for bisexuals	Maternal/paternal group	1	9.257	267	**0.002**	1	9.548	363.18	**0.002**
Full model (GLM) for parthenogens	Species	3	4.454	387.21	0.216	3	5.982	434.24	0.113
	Altitude	1	0.068	340.13	0.795	1	1.109	433.13	0.292
	SVL	1	0.613	340.06	0.434	1	0.811	432.32	0.368

		PA				ST			
Model	**Factors**	***df***	**Dev.**	**Res.dev.**	***P***	***df***	**Dev.**	**Res.dev.**	***P***
Full model (GLM) for bisexuals	Species	3	8.190	230	**0.042**	3	6.240	287	0.100
	Sex	1	0.270	229	0.604	1	0.010	287	0.923
	Altitude	1	3.210	228	0.073	1	0.300	287	0.587
	SVL	1	0.010	227	0.914	1	0.240	287	0.625
	Species × Sex	3	4.940	224	0.176	3	6.450	280	0.092
Reduced model (GLM) for bisexuals	Maternal/paternal group	1	7.079	200.53	**0.008**	1	0.151	293.40	0.697
Full model (GLM) for parthenogens	Species	3	3.267	293	0.352	3	7.426	384.83	0.059
	Altitude	1	1.907	292	0.167	1	0.000	384.83	0.995
	SVL	1	1.204	291	0.273	1	0.103	384.73	0.748

Note. df: degrees of freedom; Dev: deviance; GLM: generalized linear models; PA: amount of individuals with preanal asymmetry; Res.dev: residual deviance; ST: amount of individuals with supratemporal asymmetry; VAA: ventral scale anomalies per individual; VAI: amount of individuals with ventral asymmetry; Significant results marked in boldface.

detected in ventral scales, mainly in the chest region. Ventral scales, which we expected to best reflect the developmental stability, had most aberrations in the bisexual *D. valentini*, while the remaining bisexual species showed relatively low to moderate levels of asymmetries. In parthenogenetic *Darevskia*, the examined values of asymmetries are within the limits of their parental relatives, generally corresponding to the paternal species and being significantly higher than in the maternal species.

Different traits may be influenced independently during development (Arnold & Peterson, 2002; Van Valen,

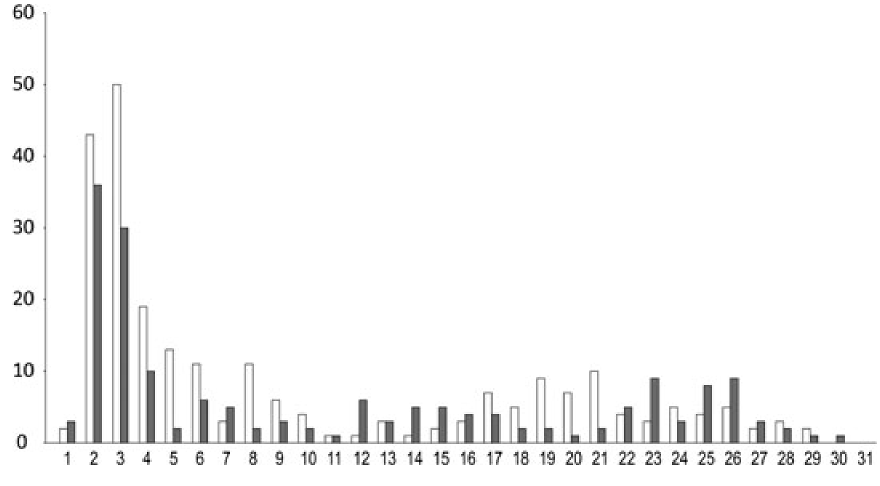

FIGURE 3 Total counts of left-sided (white columns) and right-sided ventral asymmetries (gray columns) for every row of ventral scales (1–31)

1962; Vrijenhoek & Lerman, 1982), therefore, it is possible that ventral scales may reflect different causes than preanals or supratemporals. As for the ventrals, bisexual species had a greater span of the mean count of anomalies, from 0.54 in *D. mixta* to 1.13 in *D. valentini*. Parthenogens were more conservative (0.64–0.89), which corresponds with the fact that clones are less variable in a number of morphological traits than bisexuals (Darevsky, 1967). We also cannot rule out that our results could have been influenced by a relatively small sample size in *D. valentini*. However, one of the possible explanations is the effect of higher homozygosity resulting from more frequent inbreeding in smaller populations, as was described in other lizard species (Băncilă et al., 2010; Garrido & Pérez-Mellado, 2014; Vervust, Van Dongen, Grbac, & Van Damme, 2008). *D. valentini* has the most fragmentary distribution. It inhabits isolated patches high in the mountains above 1900 m a.s.l. (Darevsky, 1967) and of the four parental species its allozyme heterozygosity indices were reported to be the lowest (MacCulloch, Fu, Murphy, Darevsky, & Danielyan, 1995; MacCulloch, Murphy, Fu, Darevsky, & Danielyan, 1997), although microsatellite data are not that clear-cut (Tarkhnishvili et al., 2017). Lazić, Rödder, and Kaliontzopoulou (2017) observed that fluctuating asymmetry increased with age notably in *D. valentini*, which also could have been caused by the fact that the individuals used in their study came from a more inbred population.

In general, our findings are in accord with other studies comparing meristic traits in naturally occurring clonal vertebrates with their bisexual relatives. Vrijenhoek and Lerman (1982) note that the smaller the population of bisexual fish of the genus *Poeciliopsis*, the more asymmetries in individuals, exceeding sometimes the values of the gynogenetic forms. Mezhzherin and Kokodii (2009) reported that both the level of fluctuating asymmetry and the occurrence of individuals with anomalies were lower in the unisexual than bisexual forms of goldfishes (*Carassius*).

An alternative explanation may involve the temperature of the environment, which may have an effect on the incubation period. *D. valentini* has the longest incubation of the four bisexual species examined. It lasts up to 3 months, while the incubation usually does not exceed 2.5 months in the remaining species (Arakelyan, Danielyan, Corti, Sindaco, & Leviton, 2011; Darevsky, 1967). Although altitude came out as an insignificant factor in our analyses, it is still a crude approximation of the climate and does not reflect the actual temperature during the ontogenesis of our specimens. Lower temperatures may increase asymmetries in some traits in lizards (Braña & Ji, 2000) and bisexuals may be even more sensitive to the temperature stress than parthenogens, as Kearney and Shine (2004a) reported for the Australian geckos *Heteronotia binoei*. This may be interpreted in terms of improved stability of enzymatic performance in heterozygotes due to the presence of enzyme variants possessing different thermal optima. However, the effect of gene dosage cannot be ruled out in parthenogenetic *Heteronotia*, due to their obligatory triploidy (Andrewartha, Mitchell, & Frappell, 2010; Kearney & Shine, 2004a).

Interpreting these results, we should consider that the parthenogens are simultaneously clones and hybrids, that is, they are genetically identical but highly heterozygous. A positive correlation between heterozygosity and developmental stability has been observed in various cases, including trouts, lizards, or mice (Alibert, Renaud, Dod, Bonhomme, & Auffray, 1994; Leary, Allendorf, & Knudsen, 1983; Shaner, Chen, Lin, Kolbe, & Lin, 2013; Young, Wheeler, & Thorgaard, 1995). Yet, heterozygosity itself is not a sufficient explanation for developmental stability, as there are cases of both outbreeding depression and stable phenotypes in various crosses. Namely in lizards, Rykena (1996) recorded increased deformities in hybrids of four distinct lizard species of the genus *Lacerta*. Contrarily, in spiny lizards (*Sceloporus*), similar or even decreased asymmetry in meristic traits was reported for crosses between species (Jackson, 1973) or chromosome races, respectively (Dosselman, Schaalje & Sites, 1998). A plausible hypothesis claims that the greater the difference between coadapted gene complexes in parents, the higher the probability of outbreeding depression in the hybrid (Clarke, 1993; Leamy & Klingenberg, 2005). This would basically correspond to the above-mentioned cases, as the parental taxa in *Sceloporus* are genetically closer than in *Lacerta* and the genetic distances between *Darevskia* species lie between those two genera (Jančúchová-Lásková et al., 2015).

Therefore, in our case, the stabilizing factor does not have to be heterozygozity itself, but rather clonal reproduction. The level of heterozygosity can vary greatly among populations of bisexual species, while being basically fixed in the clones. They would maintain a more stable rate of anomalies, regardless of their cause, for example, outbreeding depression. As the laterality tests showed, parthenogenetic *Darevskia* even displayed a more balanced distribution of ventral anomalies than bisexuals. When graphed separately, one may see that parthenogens have slightly less anomalies on the left and more on the right side of their ventrum (Figure 4). The cause of left-predominating ventral anomalies in bisexual species remains unclear. However, this pattern is shifted in the parthenogens (in a rather benign, neutral direction), which can be explained as a consequence of slight incompatibility between the parental genomes.

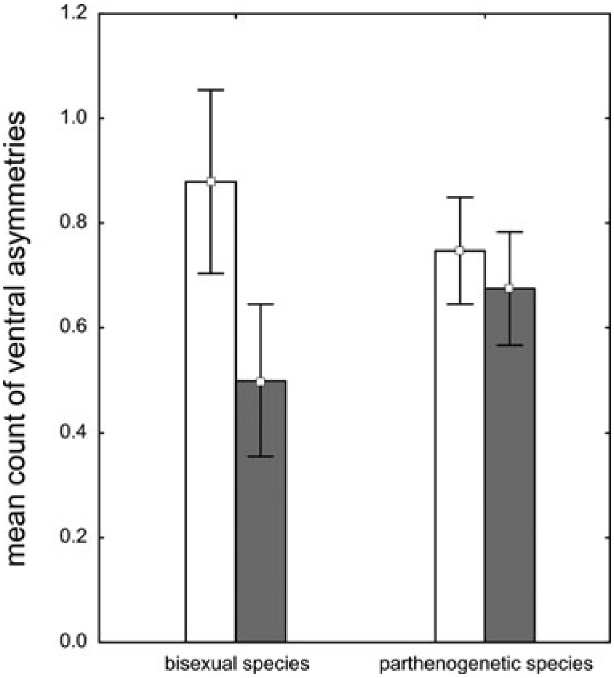

FIGURE 4 Mean counts of left-sided (white columns) and right-sided (gray columns) ventral anomalies for asymmetrical individuals in bisexual and parthenogenetic species. Whiskers denote 95% confidence interval

Asymmetries in preanals may be interpreted as a result of hybridity as well. This trait forms such patterns, which can be species-specific to a certain degree and/or numerically incompatible, for example, “odd” versus “even” configuration. Both variants occur in parental species *D. mixta*, *D. raddei*, and *D. valentini* (Darevsky, 1967; Gabelaia, Murtskhvaladze, & Tarkhnishvili, 2015). Therefore, the hybrids might be more susceptible to asymmetries in preanals, which would be a consequence of the outbreeding depression and does not necessarily mean a side effect of clonality. Similar interpretation has been given also for chin scales in parthenogenetic geckos *Nactus pelagicus* (Eckstut, Hamilton, & Austin, 2013).

In conclusion, considering the nature of our data, we can assume that parthenogenetic hybrids of the genus *Darevskia* may benefit more from high heterozygosity, which is maintained through clonal reproduction, rather than suffer from potential side effects of genetic uniformity or outbreeding depression (Jančúchová-Lásková et al., 2015). In parthenogenetic *Darevskia*, this has been reported for their ability to cope with parasites (Danielyan et al., 2008) or environmental disturbances (Murphy et al., 1997; Tarkhnishvili et al., 2010). Darevsky (1966) documented an increased amount of aberrant, nonviable embryos in parthenogenetic *Darevskia*, which already died during embryogenesis or shortly after hatching. So the severe disruption of the developmental stability does occur, but rather leads to early elimination of such individuals. We may speculate that even if detrimental consequences of hybridity or parthenogenesis occur in *Darevskia* lizards, the parthenogens survive due to a production of offspring viable enough to pass through the hard selection and/or that the phase of potential genetic decay has not yet come to pass. Recent findings even suggest that parthenogenetic lineages of *Darevskia* lizards may switch back to sexual reproduction and escape from the potential evolutionary dead-end (Danielyan et al., 2008; Spangenberg et al., 2017), therefore, there is a little evidence for their hypothetically “poorer” quality. Nonetheless, what is valid for *Darevskia* lizards does not have to be valid universally. As unisexual lineages arose independently in various groups of reptiles, each of them is unique in a way and the empirical evidence differs from case to case. One of the key challenges for the future research is to aim on the factors which influence the ontogeny and evolution of most parthenogens, but are difficult to separate. Therefore, designing a method which would distinguish individual effects of clonal reproduction, heterosis, gene dosage, outbreeding depression and environment, would be highly desirable.

ACKNOWLEDGMENTS

We are very grateful to Valentina F. Orlova for providing the material from the herpetological collections of the Zoological Museum of Moscow University, as well as for valuable comments on the manuscript. We also wish to express many thanks to the colleagues and students from the team of prof. E.G. Yavruyan in Armenia for their assistance in the field. Participation of D. F. was supported by the Czech Science Foundation, grant No. 18-15020S. Participation of P. F. was supported by the Charles University Research Centre program No. 204069.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

A. A. and D. F. conceived and designed the study. E. Y. provided resources and administered permissions. A. A., P. F., J. J-L., P. S., E. L., E. Y., and D. F. collected the material. A. A., P. F., J. J-L., P. S., E. L., and D. F. acquired the data. A. A. and D. F. analyzed and interpreted the data. A. A. drafted the manuscript. P. F., J. J-L., P. S., E. L., E. Y., and D. F. reviewed and edited the manuscript. A. A. and E. L. revised the manuscript.

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How to cite this article: Abramjan A, Frýdlová P, Jančúchová-Lásková J, et al. Comparing developmental stability in unisexual and bisexual rock lizards of the genus *Darevskia*. *Evolution & Development*. 2019;1–13. https://doi.org/10.1111/ede.12286

APPENDIX 1: MATERIAL OVERVIEW

TABLE A1 Catalogue numbers are given for museum specimens

Species	*N*	Males	Females	Locality	Catalogue number
Darevskia armeniaca	**137**		**137**		
			23	Tskhratskaro pass, Georgia	4749
			47	Sevan pass, Armenia	9504
			6	Aghavnadzor, Armenia	
			61	Dilijan, Armenia	
Darevskia dahli	**78**		**78**		
			46	Kojori, Georgia	4754, 4755
			9	Kalinino, Armenia	10277
			15 (7)	Dilijan, Armenia	2968
			3	Stepanavan, Armenia	3584
			5	Kirovakan (Vanadzor), Armenia	9505
Darevskia rostombekovi	**31**		**31**		
			5	Spitak, Armenia	9523
			13 (1)	Dilijan, Armenia	2967
			9	Göy-Göl, Azerbaijan	4175, 4176
			4	Gosh, Armenia	
Darevskia unisexualis	**72**		**72**		
			13	Hrazdan, Armenia	3576
			48	Martuni, Armenia	9530
			6 (5)	Sevan, Armenia	8150
			5	Aghavnadzor, Armenia	
Darevskia mixta	**57**	**28**	**29**		
		18	20	Bakuriani, Georgia	2970, 6028, 4752
		10	9	SW of Tbilisi, Georgia	4578
Darevskia raddei nairensis	**38**	**20**	**18**		
		12	4	Sevan, Armenia	2508
		3	4	Lchashen, Armenia	2961, 8150, 3036
			1	S of Byurakan, Armenia	9961
		1	5	Hayravank, Armenia	
		4	4	Yerevan, Armenia	
Darevskia raddei raddei	**60**	**29**	**31**		
		7	1	Geghard, Armenia	11870
		5	11	Meghri, Armenia	9959
		3	10	Shushi, Nagorno-Karabakh	9960
		12	8	Tatev, Armenia	
		2		Khosrov reserve, Armenia	
			1	Gosh, Armenia	
Darevskia portschinskii	**76**	**34**	**42**		
		26	36	Kojori, Georgia	4750, 4751
		6	5	Stepanavan, Armenia	3589, 3581, 2973
		2	1	Gosh, Armenia	
Darevskia valentini	**38**	**21**	**17**		
		5	7	Ghukasyan, Armenia	11,920
		4	3	[unknown locality], Armenia	8834
		4		Gegham ridge, Armenia	9531
			1	Sevan, Armenia	2962
			1	Mischan (Tsakhkunyants) ridge	3576
		(3)	5 (2)	Lchashen, Armenia	3582, 2974
		2		Aragats, Armenia	3062
		2		Shaghaplu, Armenia	4877
		1		Charnali river valley, Ajaria	9620

If both live and museum specimens come from the same locality, the number of live individuals is given in the brackets. Total numbers are marked in bold letters.

APPENDIX 2

TABLE A2 The incidence of scale asymmetries in eight species of Caucasian rock lizards of the genus *Darevskia*

						Counts of ventral scales anomalies					
Species group	**Species**	***N***	**VAA**	**Min**	**Max**	**0**	**1**	**2**	**3**	**4**	**5**
Maternal		155	0.555	0	3	92	46	11	6	0	0
	mixta	57	0.544	0	3	34	17	4	2	0	0
	raddei	98	0.561	0	3	58	29	7	4	0	0
Paternal		114	0.868	0	5	46	47	15	3	2	1
	portschinskii	76	0.737	0	4	34	31	9	1	1	0
	valentini	38	1.132	0	5	12	16	6	2	1	1
Parthenogenetic		318	0.802	0	5	152	103	43	16	2	2
	armeniaca	137	0.898	0	5	57	49	22	7	1	1
	dahli	78	0.821	0	5	45	14	10	7	1	1
	rostombekovi	31	0.710	0	2	13	14	4	0	0	0
	unisexualis	72	0.639	0	3	37	26	7	2	0	0

		VAI			**ST**			**PA**		
Species group	**Species**	***N***	**Count**	**%**	***N***	**Count**	**%**	***N***	**Count**	**%**
Maternal		155	63	40.6	126	39	31.0	126	13	10.3
	mixta	57	23	40.4	57	24	42.1	57	3	5.3
	raddei	98	40	40.8	69	15	21.7	69	6	8.7
Paternal		114	68	59.6	108	36	33.3	108	25	23.1
	portschinskii	76	42	55.3	73	24	32.9	73	12	16.4
	valentini	38	26	68.4	35	12	34.3	35	8	22.9
Parthenogenetic		318	166	52.2	306	104	34.0%	297	82	27.6
	armeniaca	137	80	58.4	136	48	35.3	127	21	16.5
	dahli	78	33	42.3	72	20	27.8	72	11	15.3
	rostombekovi	31	18	58.1	27	5	18.5	27	7	25.9
	unisexualis	72	35	48.6	71	31	43.7	71	22	31.0

PA: amount of individuals with preanal asymmetry; ST: amount of individuals with supratemporal asymmetry; VAA: ventral scale anomalies per individual, VAI: amount of individuals with ventral asymmetry.

V

Are parthenogenetic females less colourful than sexual ones? Evaluating UV-blue traits in the lizards of the genus *Darevskia*

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Manuscript in prep.

Are parthenogenetic females less colourful than sexual ones? Evaluating UV-blue traits in the lizards of the genus *Darevskia*

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Abstract

Sexual selection often works against the natural selection by favouring the development of conspicuous signals, raising the chances of attracting not only the potential mates, but also the predators. In lacertid lizards, ultraviolet-blue spots on their flanks and shoulders represent such a trait. Some level of correlation between male and female ornamentation is also known to exist. Therefore in the absence of the males, the females should hypothetically lose their conspicuousness. We tested this hypothesis on a complex of parthenogenetic and bisexual lizards of the genus *Darevskia*. We evaluated area, counts, UV intensity and saturation of UV-blue spots and compared the values between the clones and their bisexual progenitor species. Although some minor differences were detected, no general tendency toward higher crypsis of the parthenogens was confirmed, as their values basically corresponded to the values of sexual females. We suggest that their current phenotype rather reflects the unique combination of their parental genomes and is conserved by the clonal reproduction.

Key words

Sexual selection, parthenogenesis, UV, lizards, visual modelling

Introduction

Sexual reproduction is often coupled with sexual selection. Whether it is the male-male competition, or females choosing the most attractive males, sexual selection often leads to the development of conspicuous traits. Those may signal the quality of its bearer (so-called honest signals), typically the male, and subsequently increase the chance of reproductive success (Darwin, 1896; Andersson, 1994). On the other hand, conspicuousness also increases the risk of attracting not only potential mating partners, but also predators (Götmark, 1993; Stuart-Fox *et al.*, 2003; Husak *et al.*, 2006). In birds and reptiles, sexually selected ornaments are common also in the UV spectrum, outside our visible range. Both groups are tetrachromats, having UV sensitive cones, which enable them to use the UV channel for intraspecific communication (Gomez & Théry, 2007; Stevens & Cuthill, 2007; Mullen & Pohland, 2008; Marshall & Stevens, 2014). UV ornamentation is used as an honest signal in a number of species. Notably in lizards, the UV signals (which we perceive as blue or deep violet) are exposed directly in the perspective of the conspecifics, being mostly on the throat, flanks and the belly. Higher UV reflectance is related to fight success and higher female preferences in European green lizard, *Lacerta viridis* (Bajer *et al.*, 2011; Molnár *et al.*, 2012), sand lizard, *Lacerta agilis* (Olsson, Andersson, & Wapstra, 2011) or flat lizards *Platysaururs broadleyi* (Whiting *et al.*, 2006). Sexual dichromatism in the UV-blue coloration, suggesting similar function, was reported in *Gallotia galloti* (Bohórquez-Alonso *et al.*, 2018), *Timon lepidus* (Font, Pérez I De Lanuza, & Sampedro, 2009) or lizards of the genus *Podarcis* (Pérez i de Lanuza & Font, 2010). This applies also to Caucasian rock lizards of the genus *Darevskia*. The genus is remarkable for seven obligatory parthenogenetic species, which arose from interspecific hybridization of at least four bisexual species, two of them being paternal and two maternal ancestors (Moritz *et al.*, 1992; Freitas *et al.*, 2016; Tarkhnishvili, Murtskhvaladze, & Anderson, 2017). *Darevskia* lizards cluster into three main groups, with paternal species belonging to one group and maternal species to another one (Murphy *et al.*, 2000).

As the parthenogens lack males, we decided to explore whether the absence of sexual selection affects the extent or intensity of their UV-blue coloration. There are several explanations addressing the positive correlation between female and male ornamentation. Firstly, female ornamentation can be actively selected for by the males, so the females can be the target of sexual selection as well (Amundsen, 2000). This is imaginable in the *Darevskia* lizards, as the males actively pursue the females during the mating period (Darevsky, 1967).

Secondly, the conspicuous traits may correlate between the sexes due to shared genetic basis (Potti & Canal, 2011). Therefore we may presume that when the sexual selection is missing, natural selection on higher crypsis would take action. Significant effects of natural selection on the colouration of parthenogens would be especially interesting, as it would mean that even clonal organisms react to selection pressures despite being genetically uniform. For this purpose, we examined the extent and intensity of UV-blue spots in four parthenogenetic species (*D. armeniaca, D. dahli, D. rostombekovi* and *D. unisexualis*) and their respective paternal (*D. portschinskii, D. valentini*) and maternal (*D. mixta, D. raddei/D. nairensis*) and one unrelated (*D. caucasica*) species.

Material and methods

The material comprised 316 lizards of 9 species belonging to the complex of bisexual and parthenogenetic species. One species, *D. caucasica*, is unrelated to the parthenogens, but belongs to the same phylogenetic group as the maternal species *D. mixta* and *D. raddei/D. nairensis* (Murphy *et al.*, 2000). Most individuals were live except all *D. mixta* and part of *D. valentini*, which came from the collections of Zoological Museum in Moscow. The lizards were either captured in the wild during the start of their breeding season in May and June, or obtained from a private breeder during the same period. Maternal species: *D. mixta* – 14 males, 24 females; *D. nairensis* – 16 males, 12 females; *D. raddei* – 20 males, 11 females. Paternal species: *D. portschinskii* – 21 males, 21 females; *D. valentini* – 12 males (9 preserved specimens), 2 females. Unrelated: *D. caucasica* – 14 males, 17 females. Parthenogenetic: *D. armeniaca* n = 76; *D. dahli* n= 25; *D. rostombekovi* n = 15; *D. unisexualis* n = 16. For better precision, we decided to discriminate between *D. nairensis* and *D. raddei* in this study. Although conspecific, each of the forms (subspecies, populations) contributed to formation of distinct parthenogens; *D. unisexualis* is related to *D. nairensis* (Freitas *et al.*, 2016), while *D. rostombekovi* is probably related to *D. raddei* (Fu, Murphy, & Darevsky, 2000b). List of species, sexes and localities is given in Appendix 1A.

We aimed on both quantitative and qualitative parameters of UV-blue spots in two body regions – the ventrolateral row of outer ventral scales (henceforth OVS) and the shoulder. We assessed the quantitative parameters for OVS, which included percentage of the blue area, count of the blue spots and the relative spot size, expressed as blue area divided by the number of spots. Qualitative parameters were measured for both OVS spots and shoulder spots and included the intensity of UV and the mean saturation. Only smaller subsets of individuals were available for qualitative measurements. For details see Appendix 1B and 1C.

Image acquisition and processing

For quantitative measurements, we scanned each lizard with an Epson GT-S640 scanner at a 600 dpi resolution. We cut out both left and right rows of OVS in Adobe Photoshop CS6 and calculated proportional areas of the blue colour with Barvocuc software (Rádlová, Viktorin, & Frynta, 2016) (fig. 1). The hue angle for blue was set to 175-275° and the tolerance of grey to 8%. The blue spots were partially faded in the museum specimens, but still recognizable by the human eye. The specimens were photographed with Nikon E4500 digital camera. However, after the OVS the faded spots had to be restored digitally, to make them “visible” to

Barvocuc software. In order to make the estimation as objective as possible, it was done independently by two of the authors and their conclusions were compared. The number of blue spots was counted manually for each side of a lizard, comparing the outputs of Barvocuc with the original scans. Number of scales in each OVS row was recorded as well, as it is a sex dependent trait (Darevsky, 1967). Counts from both sides were then averaged for each lizard.

The qualitative traits were measured using UV photography and Multispectral Image Calibration and Analysis Toolbox (MICA) v2 (van den Berg *et al.*, 2019), a freely available plug-in running on ImageJ platform (Schneider, Rasband, & Eliceiri, 2012). Each lizard was photographed from its lateral side through UV/IR cut filter, transmitting visible light 400-700 nm, and then UV pass Baader U-Venus filter, transmitting 300-400 nm (fig. 2). The camera used was Samsung NX1000 adapted for UV photography (according to the instructions by J. Troscianko, available at https://www.jolyon.co.uk/2014/07/full-spectrum-nx1000/), with 35mm Novoflex Noflexar lens. Lizards were placed 16 cm from the lens and illuminated by Iwasaki ColorEyeArc bulb with UV blocking coating removed. The lamp was set 20 cm above the lens. White PTFE (teflon) plates were put around the lizard to eliminate undesirable shadows. Photographs were calibrated against a white PTFE tape having flat 99% reflectance from 300 to 700 nm, which was checked by spectrophotometer against white WS-1 diffuse reflectance standard. The photographs were further processed with the MICA toolbox according to the methodology of (Troscianko & Stevens, 2015; van den Berg *et al.*, 2019).

Three different OVS blue spots were selected on each lizard, where possible, and treated as one region of interest. Where more than one blue shoulder spots were present, we selected the largest one. For visual modelling, we used the photoreceptor data for *Podarcis muralis*, cone abundance ratio UVS:SWS:MWS:LWS 1:2:5:9, Weber fraction 0.05 (Martin *et al.*, 2015). Each multispectral stack was then converted to RNL (receptor noise limited) XYZ chromaticity system. XYZ stand for the three axes corresponding to opponent channels in a tetrachromat's vision. X, Y and Z axes represent 'red-green' (LW:MW), 'yellow-blue' ((LW+MW):SW) and 'visible-ultraviolet' ((LW+MW+SW):UV) opponency, respectively (van den Berg *et al.*, 2019). Each colour is therefore described by three coordinates. We used two parameters as measures of the UV-blue spots' quality: 1) the intensity or dominance of UV signal expressed in Z axis values and 2) saturation of the colour, expressed as mean Euclidean distance from the achromatic point of the colour space to the colours measured within a particular region of interest.

Statistical analysis

We checked data for normality and applied transformations where needed (*arcsine*: percentage of blue area; *square root*: blue spot counts; *log*: spot size, spot saturation). We used a linear model (LM) to determine the effects of species and sex in bisexual species. In females, sexual and parthenogenetic, we used LM to detect the effect of species and carried out also generalized least squares (GLS) model with reproduction mode as predictor and species as random factor. The blue spots count was weighted by the number of scales in the OVS row. Then we checked particular differences between parthenogens and their respective parental species of both sexes with planned post hoc comparisons, using Unequal N HSD test. All calculations were performed with R v3.5.0 and Statistica 8 softwares.

Results

In bisexual species, linear models revealed significant effect of species and sex in four traits: blue area, blue spot count, OVS spot size and shoulder spot UV intensity (table 1). Effect of species alone was significant also in OVS saturation and shoulder spot saturation, while no effect was proven in OVS UV intensity. Males expressed higher values than females in most parameters (fig. 3&4). Comparisons within the females set, comprising both sexual and parthenogenetic species, revealed significant ‘species’ effect in all cases. GLS model, however, detected no effect of parthenogenesis as such. In planned post hoc comparisons, parthenogenetic species showed no significant differences against females from either parental group. The only exception was *D. armeniaca*, which had larger area of blue colour (11.65% ± SE 0.43; $p < 0.001$), more blue spots (9.8 ± SE 0.25; $p < 0.001$) and bigger average spot size (1.2 ± SE 0.04; $p = 0.019$) than females of its maternal parent *D. mixta* (blue area: 2.94 % ± SE 0.55; spot count: 4.0 ± SE 0.57; spot size: 0.75 ± SE 0.19) and closely related parthenogenetic *D. dahli* (blue area: 1.81 % ± SE 0.26; spot count: 3.74 ± SE 0.58; spot size: 0.55 ± SE 0.21; $p < 0.001$ in all three parameters). Blue shoulder spots were also less intense in UV in *D. dahli* (-2.0 ± SE 1.18) than in *D. armeniaca* (6.56 ± SE 1.05; $p = 0.001$) (fig. 4). In D. *nairesis/D.raddei* complex, there was a substantial effect of population for both blue area ($F = 15.837$, $p < 0.001$) and spot count ($F = 12.354$, $p < 0.001$), with the population of *D. nairensis* from Hayravank having higher values than the Yerevan population or the populations of *D. raddei* (fig. 5).

Table 1. Linear model and GLS results for the quantitative and qualitative parameters of the UV-blue spots. Abbreviations; *outer ventral scales,* OVS:BA – blue area of outer ventral scales, OVS:BSC – blue spot count, OVS:SS – spot size, OVS:UV – UV channel response, OVS:Sat – saturation; *shoulder spots,* Sh:UV – UV channel response, Sh:Sat – saturation. Significant values are marked in boldface.

	Df	OVS:BA		OVS:BSC		OVS:SS	
		F	p	F	p	F	p
Bisexual species							
Species	5	19.832	<**0.001**	11.361	<**0.001**	5.920	<**0.001**
Sex	1	68.226	<**0.001**	30.925	<**0.001**	23.270	<**0.001**
Species:sex	5	2.912	**0.015**	2.400	**0.039**	1.250	0.288
Females							
Species	9	36.805	<**0.001**	10.930	<**0.001**	11.329	<**0.001**
Reproduction mode (GLS)	1	0.054	0.816	0.019	0.891	0.197	0.657
ρ value		0.619		0.323		0.150	

	Df	OVS:UV		OVS:Sat		Sh:UV		Sh:Sat	
		F	p	F	p	F	p	F	p
Bisexual species									
Species	2	3.043	0.064	7.455	**0.003**	4.719	**0.017**	4.093	**0.028**
Sex	1	3.515	0.072	3.367	0.078	11.348	**0.002**	1.835	0.187
Species:sex	2	2.267	0.123	1.537	0.233	1.790	0.186	0.124	0.884
Females									
Species	6	6.366	<**0.001**	9.207	<**0.001**	5.229	<**0.001**	4.190	**0.002**
GLS: Reproduction mode	1	0.002	0.962	0.456	0.502	0.156	0.695	0.201	0.656
ρ value		0.560		0.582		0.405		0.373	

Discussion

We have confirmed a pronounced sexual dimorphism in UV-blue traits within the bisexual species of *Darevskia* lizards in both quantitative and qualitative parameters. In general, the parthenogenetic species were not proven to be significantly different from bisexual species in either trait. Though, some tendencies can be observed in relation towards their maternal and paternal ancestors. In quantitative traits, the most prominent parthenogen was *D. armeniaca*, exceeding its matrilinear ancestor *D. mixta* in the extent of blue area as well as in the number of blue spots. In the size of OVS spots, it even resembled males of its both paternal and maternal progenitor species, *D. valentini* and *D. mixta*, respectively. According to an alternative hypothesis, the maternal ancestor of *D. armeniaca* is *D. dahli* (Tarkhnishvili *et al.*, 2017), which itself is a hybrid of *D. mixta* and *D. portschinskii*. This would, however, interfere little with the interpretation, considering the fact that the matrilinear genome of *D. mixta* would be probably passed to *D. armeniaca* without major changes due to clonal reproduction of *D. dahli*. Of course, one should keep in mind that all individuals of *D. mixta* used in this study were alcohol-fixed. When compared to other sexual species, e.g. the related *D. raddei* or unrelated *D. caucasica*, *D. mixta* values can be found convincing. Still, there is a certain probability that the count and extent of blue spots was in fact underestimated, as colours fade away in preserved specimens. Therefore the significant difference between *D. armeniaca* and *D. mixta* females can be an artefact.

D. unisexualis had values very similar to *D. armeniaca*, significant difference has not been detected in any of the traits studied. The same can be told about the mutual resemblance of *D. dahli* and *D. rostombekovi*. As each pair has a different paternal progenitor, it is possible that at least in the case of *D. armeniaca* and *D. unisexualis*, the parental genome of *D. valentini* has certain dominant effect. Not only have both parthenogens similar (and higher) values in both quantitative and qualitative traits (Fig. X), but they inherited also larger size from *D. valentini* (Darevsky, 1967). This would be in partial accordance with our previous findings, which suggest that the parthenogens are closer to their paternal species regarding the amount of scalation asymmetries (Abramjan *et al.*, 2019).

D. dahli and *D. rostombekovi* are least colourful among the parthenogens, whether speaking about the blue area, saturation or UV intensity. On the other hand, similar values can be found also in females of their parental species *D. mixta*, *D. raddei* and *D. portschinskii*. The qualitative parameters of the UV-blue spots therefore do not seem to differ between sexual and clonal females and the most pronounced changes affect the extent (and not the count) of the OVS blue spots (*D. dahli versus D. armeniaca*).

There is also a notable fact concerning the bisexual *D. nairensis*. The high variability in this species was caused mainly by the Hayravank population (Lake Sevan shore, 1900 m a.s.l., where both sexes were notably more colourful compared to Yerevan population (900 m a.s.l). The latter is in fact similar in its values to populations of *D. raddei* (fig. 5). This would support the initial presumption that the sexual selection may boost conspicuousness in both sexes simultaneously. There are several possible explanations, why the Hayravank population is so colourful. First, the UV irradiance is higher in high altitudes, therefore the lizards might take advantage of this by maximizing the area of UV reflective spots. Second, the Hayravank locality is extensively covered by bright orange lichens, against which the UV-blue colouration must be highly contrasting. Though, it is also possible, that sexual selection is not involved so much and other causes are behind the phenomenon, e.g. founder effect.

The question whether the absence of sexual selection could eventually decrease the conspicuousness in parthenogens, however, remains. Given the fact that both drab (*D. dahli*) and relatively colourful (*D. armeniaca*) species exist among the parthenogens, but stay within the limits of their parental species' diversity, other interpretations of their colouration seem to be more likely. As each of the clonal species originated from a unique combination of parental species, their resultant phenotypes may rather reflect the dominance of the respective genomes and stay more or less conserved by the clonal reproduction since their origination. Of course, certain variability has been reported in parthenogens as well. Part of *D. dahli* from Dilijan has bright yellow bellies, whereas their typical ventral coloration is dull cream yellow (Arakelyan *et al.*, 2011). There is also a colour morph within the population of *D. armeniaca* from Marmarik river valley in central Armenia, having striped pattern on its back instead of a spotted one (Fu *et al.*, 2000a). We do not have much knowledge on the genetics of these colour morphs as such. Though, recent genetic studies have confirmed presence of several clones within *D. armeniaca* (Girnyk *et al.*, 2018), *D. dahli* (Vergun *et al.*, 2014; Tarkhnishvili *et al.*, 2017) and *D. rostombekovi* (Ryskov *et al.*, 2017) which probably did not result from random mutations and rather suggest multiclonal origin of these parthenogens. This would be a potential field for further investigations. Firstly, there might be certain phenotypic differences between the clones of a single parthenogenetic species, which would get unnoticed when pooling them together. These could possibly be informative about the phenotypes involved in past hybridizations. Secondly, revealing potential epigenetic effects of the environment would be more accurate when aiming on a single clone. Individuals of *D. armeniaca* and *D. dahli*, introduced in 1960s to central Ukraine, showed shifted values in their morphology (mostly size) compared to their original populations in the Caucasus and also melanism, unknown from native populations, was reported (Darevsky, 1967; Nekrasova & Kostiushyn, 2016). Differences in colouration in response to varying environment are therefore expectable. Due to their limited origins, but relatively large distributions, the parthenogens had to expand. They colonized intermediate habitats, where natural conditions are between those preferred by their parental species, and even outcompeted the bisexual progenitors in sympatry zones (Tarkhnishvili *et al.*, 2010; Freitas *et al.*, 2016). Some authors use the 'weed' metaphor, regarding parthenogens as weeds settling in disturbed or suboptimal habitats (Wright & Lowe, 1968). Because the range of optimal habitats is always larger than the actual distribution (Tarkhnishvili *et al.*, 2010), it would be interesting to test, whether the parthenogens prefer localities with backgrounds they match better, or whether they are able to adapt to various backgrounds. Our study aimed solely on the sexually selected UV-blue spots. However, a complex evaluation of the overall pattern, including the colouration of the back, belly and the lateral flanks, with regard to the natural background, would be interesting for the future research.

Acknowledgements

We are very grateful to Valentina F. Orlova for kindly providing the material from the Zoological Museum in Moscow, Petra Frýdlová, Jitka Jančúchová-Lásková and Petra Hnidová (Suchomelová) for assistance with collecting and processing the material, Marcel Honza for auxiliary spectrophotometric measurements, Michal Šulc for checking the white reflectance standards with a spectrophotometer, and to Valentina Azaryan for helping us with administrative and field issues. This paper is dedicated to the memory of prof. Eduard G. Yavruyan, to whom we pay our greatest gratitude for making our long term scientific collaboration and field expeditions possible.

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Figure 1. Examples of OVS rows used for the analysis of quantitative parameters of the UV-blue spots. Each pair shows the original scan and the output from Barvocuc software. In *D. mixta*, the middle row shows the digitally reconstructed pattern; a. *D. caucasica*, male, b. *D. caucasica*, female, c. *D. mixta* male, d. *D. mixta*, female, e. *D nairensis*, male, f. *D. nairensis*, female, g. *D. portschinskii*, male, h. *D. portschinskii*, female, i. *D. raddei*, male, j. *D. raddei*, female, k. *D. valentini*, male, l. *D. valentini*, female, m. *D. armeniaca*, n. *D. dahli*, o. *D. rostombekovi*, p. *D. unisexualis*. a-l: bisexual species, m-o: parthenogenetic species. Scale not preserved.

Figure 2. Visible and UV photo of a male *D. nairensis*, used for the analysis of qualitative parameters of shoulder and OVS spots.

Figure 3. Quantitative parameters of OVS spots by species and sex. Dark grey: males, light grey: females, white: parthenogens. Box plots – middle point: median, box:1st-3rd quartile, whiskers: non-outlier range, dots: outliers. Columns – average, whiskers: 95% confidence interval.

Figure 4. Qualitative parameters of OVS and shoulder spots by species and sex. Dark grey: males, light grey: females, white: parthenogens. Middle point: median, box:1st-3rd quartile, whiskers: non-outlier range, dots: outliers.

Figure 5. Population differences in the extent of OVS blue area in *D. nairensis* and *D. raddei*. Middle point: median, box:1st-3rd quartile, whiskers: non-outlier range, dots: outliers.

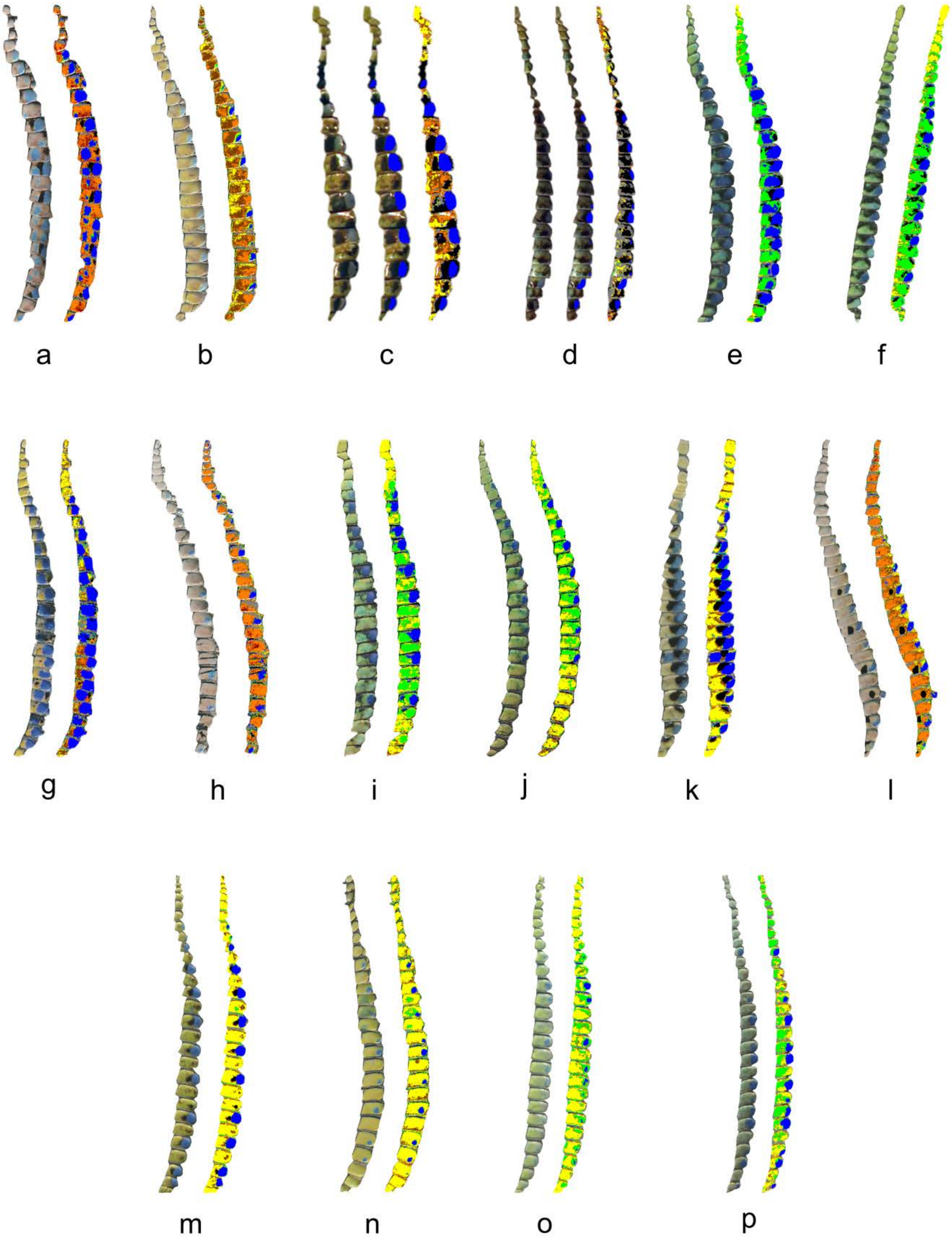

Figure 1

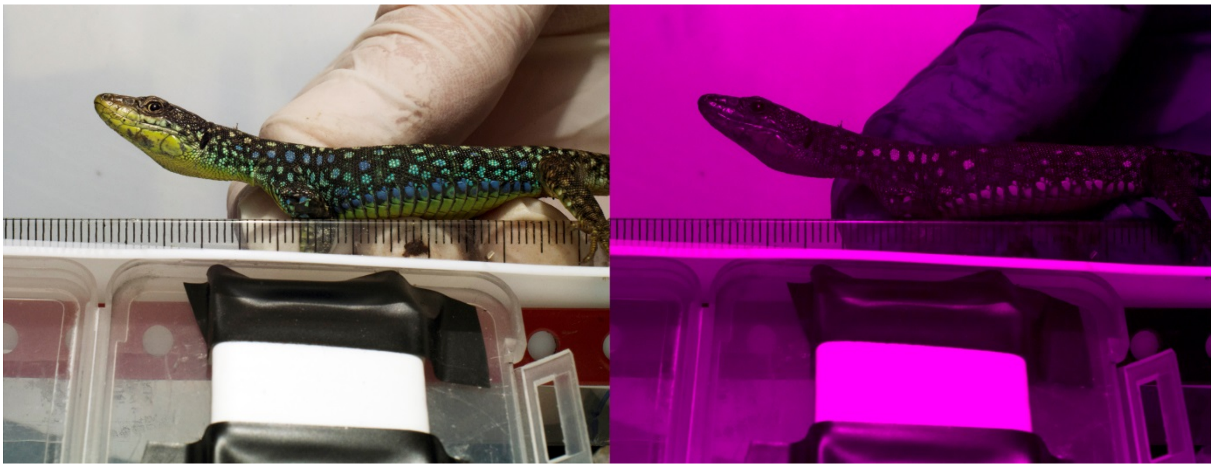

Figure 2

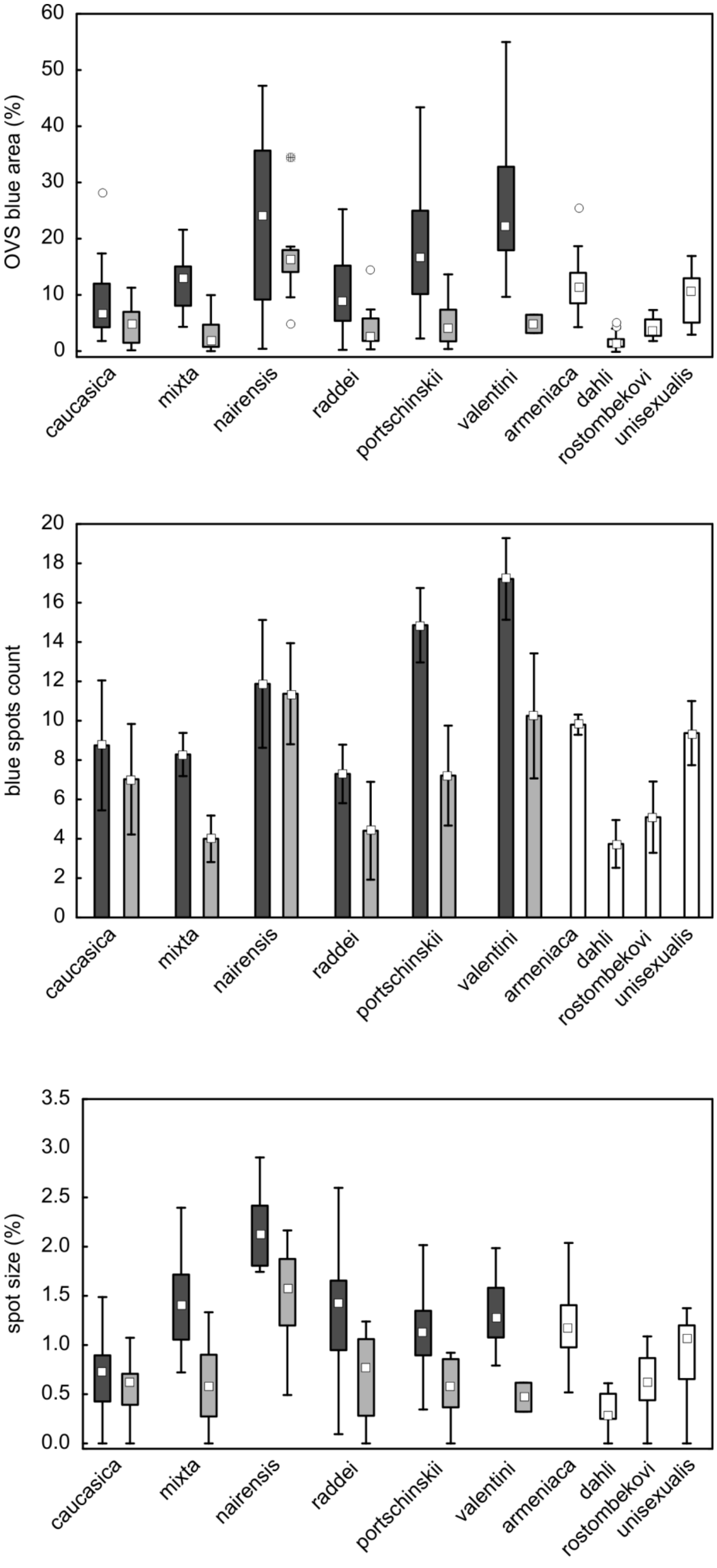
OVS blue area (%)
0
10
20
30
40
50
60
caucasica
mixta
nairensis
raddei
portschinskii
valentini
armeniaca
dahli
rostombekovi
unisexualis
blue spots count
0
2
4
6
8
10
12
14
16
18
20
caucasica
mixta
nairensis
raddei
portschinskii
valentini
armeniaca
dahli
rostombekovi
unisexualis
spot size (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
caucasica
mixta
nairensis
raddei
portschinskii
valentini
armeniaca
dahli
rostombekovi
unisexualis

Figure 3

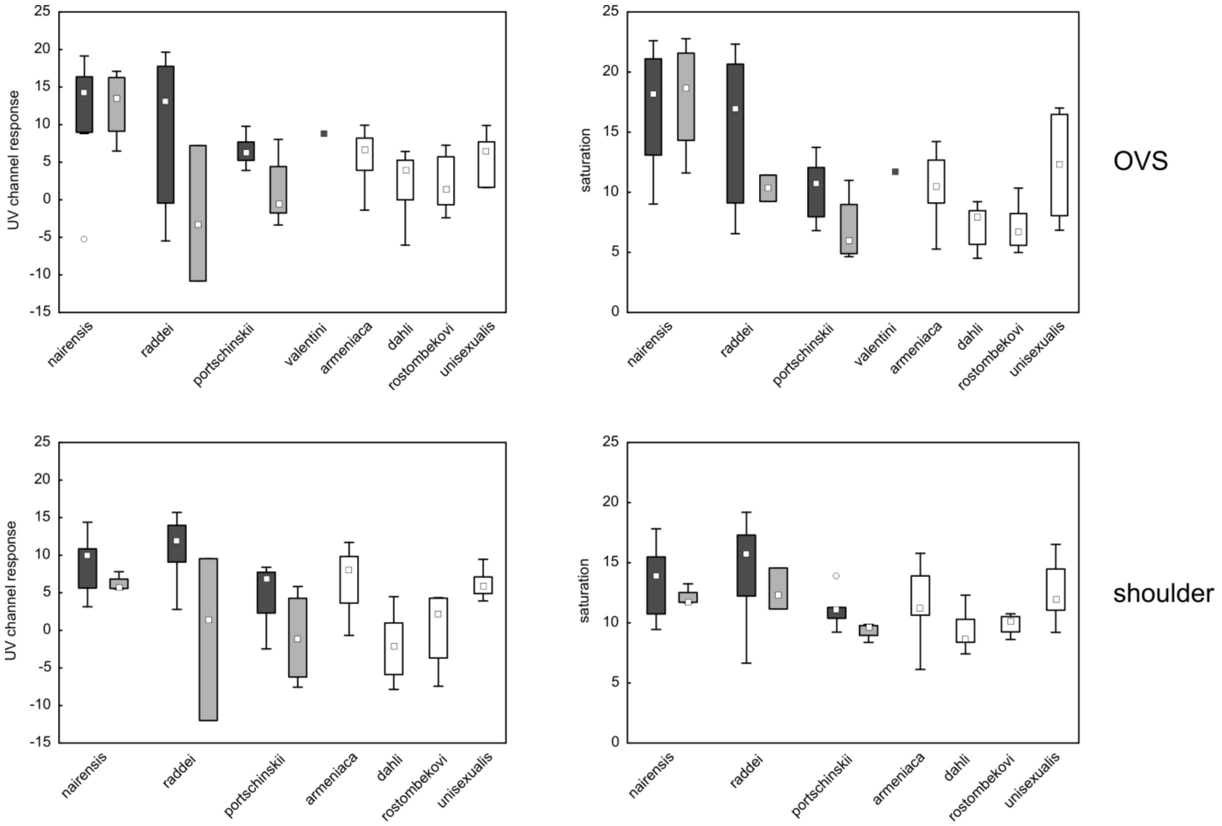
UV channel response
saturation
OVS
shoulder
25
20
15
10
5
0
-5
-10
-15
nairensis
raddei
portschinskii
valentini
armeniaca
dahli
rostombekovi
unisexualis

Figure 4

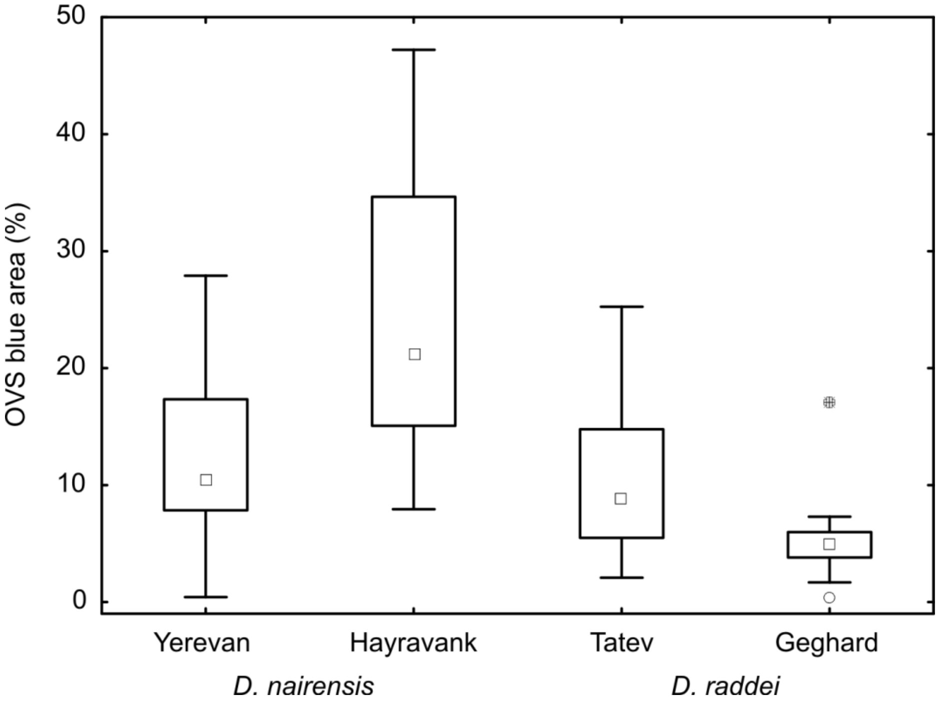
OVS blue area (%)
0
10
20
30
40
50
Yerevan
Hayravank
Tatev
Geghard
D. nairensis
D. raddei

Figure 5

Appendix 1

A. List of species, localities and sexes used for quantitative traits evaluation.

species	**locality**	**males**	**females**
armeniaca	Dilijan	0	55
armeniaca	Aghavnadzor	0	5
armeniaca	Lchashen	0	3
armeniaca	Makaravank	0	1
armeniaca	Haghartsin	0	4
armeniaca	Hankavan	0	8
caucasica	Kazbek	9	12
caucasica	Tusheti	5	5
dahli	Kojori	0	12
dahli	Dilijan	0	13
mixta	Bakuriani*	7	18
mixta	SW of Tbilisi*	7	6
nairensis	Hayravank	9	8
nairensis	Yerevan	7	4
portschinskii	Kojori	12	15
portschinskii	Gori	5	5
portschinskii	Gosh	4	1
raddei	Garni	1	0
raddei	Geghard	7	3
raddei	Gosh	0	2
raddei	Tatev	12	6
rostombekovi	Dilijan	0	1
rostombekovi	Gosh	0	6
rostombekovi	Haghartsin	0	8
unisexualis	Sevan	0	16
valentini	Ghukasyan*	2	0
valentini	Lchashen	3	2
valentini	Armenia [unspecified]*	3	0
valentini	Gegham*	3	0
valentini	Ajaria*	1	0

*material from the collections of the Zoological Museum in Moscow

B. Subset of individuals used for qualitative traits evaluation (OVS)

species	**locality**	**males**	**females**
armeniaca	Haghartsin	0	4
armeniaca	Hankavan	0	8
armeniaca	Lchashen	0	2
dahli	Dilijan	0	6
dahli	Kojori	0	8
nairensis	Hayravank	7	4
nairensis	Yerevan	3	0
portschinskii	Kojori	9	12
portschinskii	Gosh	1	0
raddei	Garni	1	0
raddei	Geghard	7	3
rostombekovi	Gosh	0	3
rostombekovi	Haghartsin	0	7
unisexualis	Sevan	0	8
valentini	Lchashen	1	0

C. Subset of individuals used for qualitative traits evaluation (shoulder spots)

species	**locality**	**males**	**females**
armeniaca	Haghartsin	0	4
armeniaca	Hankavan	0	8
armeniaca	Lchashen	0	2
dahli	Dilijan	0	3
dahli	Kojori	0	8
nairensis	Hayravank	7	4
nairensis	Yerevan	3	0
portschinskii	Kojori	5	4
raddei	Garni	1	0
raddei	Geghard	6	3
rostombekovi	Gosh	0	1
rostombekovi	Haghartsin	0	3
unisexualis	Sevan	0	8