Ultraviolet reflectance of male nuptial colouration in sand lizards (*Lacerta agilis*) from the Pyrenees

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Abstract. The evolutionary significance of lacertid colourations is relatively unexplored. However, several studies have demonstrated signalling by means of bright green colouration in Swedish *Lacerta agilis* males during the breeding season. Unfortunately, most of these studies have been based on human colour perception that differs in several ways from that of lizard. An important difference between human and lizard colour vision is the presence of an ultraviolet (UV) sensitive cone in lizards. The available evidence suggests that male sand lizards colorations do not reflect UV wavelengths, at least in Swedish populations. However, this study, based on objective (spectrophotometric) measures of Pyrenean *L. agilis*, revealed a secondary reflectance peak in the UV in male green colouration. This secondary peak increases sexual dichromatism and male conspicuousness. Moreover, it could have a social signalling function as do similar UV reflecting patches in other lizard species.

Lacerta agilis (Linnaeus, 1758) has one of the largest distribution ranges of all lacertids, extending from Lake Baikal to the Pyrenees and from S Sweden to N Greece (Bischoff, 1988). It is also one of the best studied lacertid species and there is an extensive body of literature on its morphology, physiology, ecology, and behaviour (i.e. approximately 150 papers are cited in Bischoff, 1984).

Sand lizards are sexually dichromatic throughout most of their distribution range (Arnold and Ovenden, 2002): during the breeding season females are uniformly grayish-brown whereas males are brownish mid-dorsally and display patches of bright green colouration ventrally and laterally. During the mating season males fight for access to mates. After copulation, males show mate guarding behaviour and reject other males. During contests, males approach rivals with a dorso-ventrally flattened body, displaying lateral green nuptial colouration (Kitzler, 1941; Olsson, 1992, 1994a; Weyrauch, 2005).

Pivotal work by Olsson and colleagues with sand lizards from a population in the Swedish west coast has shown that the size and/or brightness of the green patches can act as an honest signal of fighting ability (Olsson, 1994a; Olsson and Silverin, 1997) or as a cue to male genetic quality (Olsson et al., 2005a, b). The area covered by the green patches increases allometrically with body size and its pigment saturation (i.e. ‘chroma’) correlates both with body mass and with an index of body condition (Olsson, 1993). Males with artificially enlarged lateral green patches are more likely to win staged encounters in the laboratory (Olsson, 1994b) and have higher mating success in the field (Anderholm et al., 2004). The size of a male’s lateral green patches is negatively correlated with male mate guarding duration (Olsson et al., 2000), and males with more diverse MHC genotype have more and brighter nuptial colouration (Olsson et al., 2005a). It seems that females are attracted to males with MHC genotype different from their own (Olsson et al., 2003) and respond to colour-enhanced males by adjusting maternal effort and the sex ratio of their offspring (Olsson et al., 2005b).

Unfortunately, most of the above studies have been conducted using methods and techniques optimized for human vision (e.g. Munsell’s colour charts to calculate chroma, stan-

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dard colour photography, dyes mixed to match by eye the lizards' natural colouration, etc.) that do not take into account potentially important differences between our visual perception and that of other animals. Many lizards, for example, are capable of seeing into the ultraviolet (UV) range of wavelengths and display elaborate UV reflectance patterns that appear to be used for intraspecific signalling (Fleishman et al., 1993; LeBas and Marshall, 2000; Macedonia et al., 2000, 2003; Blomberg et al., 2001; Fleishman and Persons, 2001; Macedonia, 2001; Stoehr and McGraw, 2001; Thorpe and Richard, 2001; Thorpe, 2002; Thorpe and Stenson, 2003; Font and Molina-Borja, 2004; Molina-Borja et al., 2006; Stapley and Whiting, 2006; Whiting et al., 2006). Only two recent studies have relied on objective techniques (i.e. reflectance spectrophotometry) to asses the colouration of sand lizards or of paints used to manipulate their colour patches (Anderholm et al., 2004; Olsson et al., 2005b). Both studies cite unpublished spectrophotometry data showing that Swedish sand lizards lack reflectance in the ultraviolet spectrum (Olsson and Andersson, unpubl. data). Here we use reflectance spectrophotometry to show that, in contrast to the Swedish lizards, the green patches of males from a sand lizard population in the Pyrenees exhibit a distinct reflectance peak in the UV.

Spectral data were gathered for six adult males (mean SVL ± SD = 76 ± 2.04 mm) and one female (SVL = 74 mm). All lizards were captured with a pole and noose in May 2005 in Serra del Cadí, eastern Pyrenees, 42°20′45″ N, 1°54′55″ E (for a description of the population and study site see Amat et al., 2000, 2003). Lizards were held in cloth bags after data collection, and all were released unharmed within 24 h at their location of capture. Experimental techniques and protocols have been described in detail elsewhere (Font and Molina-Borja, 2004; Molina-Borja et al., 2006). We therefore only present a summary here. We collected reflectance spectra using an Ocean Optics USB2000 portable diode-array spectrometer and a PX-2 xenon strobe light source, both from Ocean Optics B.V. (Duiven, The Netherlands). Spectra were recorded in 0.37-nm steps from 200 to 850 nm and expressed as per cent of light reflected relative to a Spectralon white diffuse reflectance standard. Measurements were taken with a reflectance probe (R200-7, Ocean Optics) held at a 90° angle to and 5 mm from the lizard's skin. A dark current and white standard reference spectrum were taken every 10 min during measurement of lizard colour patches. Spectra were obtained from the following body parts: head (dorsal, anterior to parietal eye), gular area (centre), lateral patches, dorsum (midpoint along the vertebral column), ventrum (centre), leg (outer, upper thigh), and tail (dorsal, slightly distal to base). In addition, we obtained spectra of samples of natural backgrounds that are present in *L. agilis* habitat: green vegetation and soil. For each measurement the spectrometer averaged 20 spectra that were graphed using OOIBase32 software from Ocean Optics. Integration time was set at 55 ms using data-smoothing level 10. All measurements were taken in a darkened room to minimize interference from external light sources.

The mid-dorsal stripe of male sand lizards produces, as does most of the body surface of females, a relatively flat spectrum with negligible reflectance below 400 nm (fig. 1). In contrast, the lateral and ventral surfaces of males show dual reflectance peaks in the green (primary peak) and UV (secondary peak) regions of the spectrum. The primary peak, responsible for the green hue perceived by humans, has the maximum of reflectance near 530 nm, while the UV peak has its maximal reflectance around 350 nm (fig. 1). The green peak is brighter than the UV peak. The ventral surface is brighter than scales of the lateral patches. In the belly, reflectance of the green peak is approximately 58%, while the UV peak reflects 36 % of incident light. On the lateral patches, green peak reflection is 33% and UV peak reflection is 15%.

Our results show that the nuptial colouration of male sand lizards from the Pyrenees is highly UV-reflective. Other lacertids, like *Gallotia* (Thorpe and Richard, 2001; Font and Molina-Borja, 2004; Molina-Borja et al., 2006) and several species of *Podarcis* (unpubl. data) have colour patches that reflect ultraviolet wavelengths. In fact, the green patches of male sand lizards produce spectra very similar to those recorded from the ventrolateral green ocelli (eyespots) of *Gallotia atlantica* from Fuerteventura (Canary Islands), which also have a secondary peak at around 360 nm (see Fig. 5D in Font and Molina-Borja, 2004). Colour patches with multiple reflectance peaks, including a secondary UV peak that is confined to or extends into the UV region have also been reported in the dewlaps of *Anolis* lizards (Macedonia, 1999,

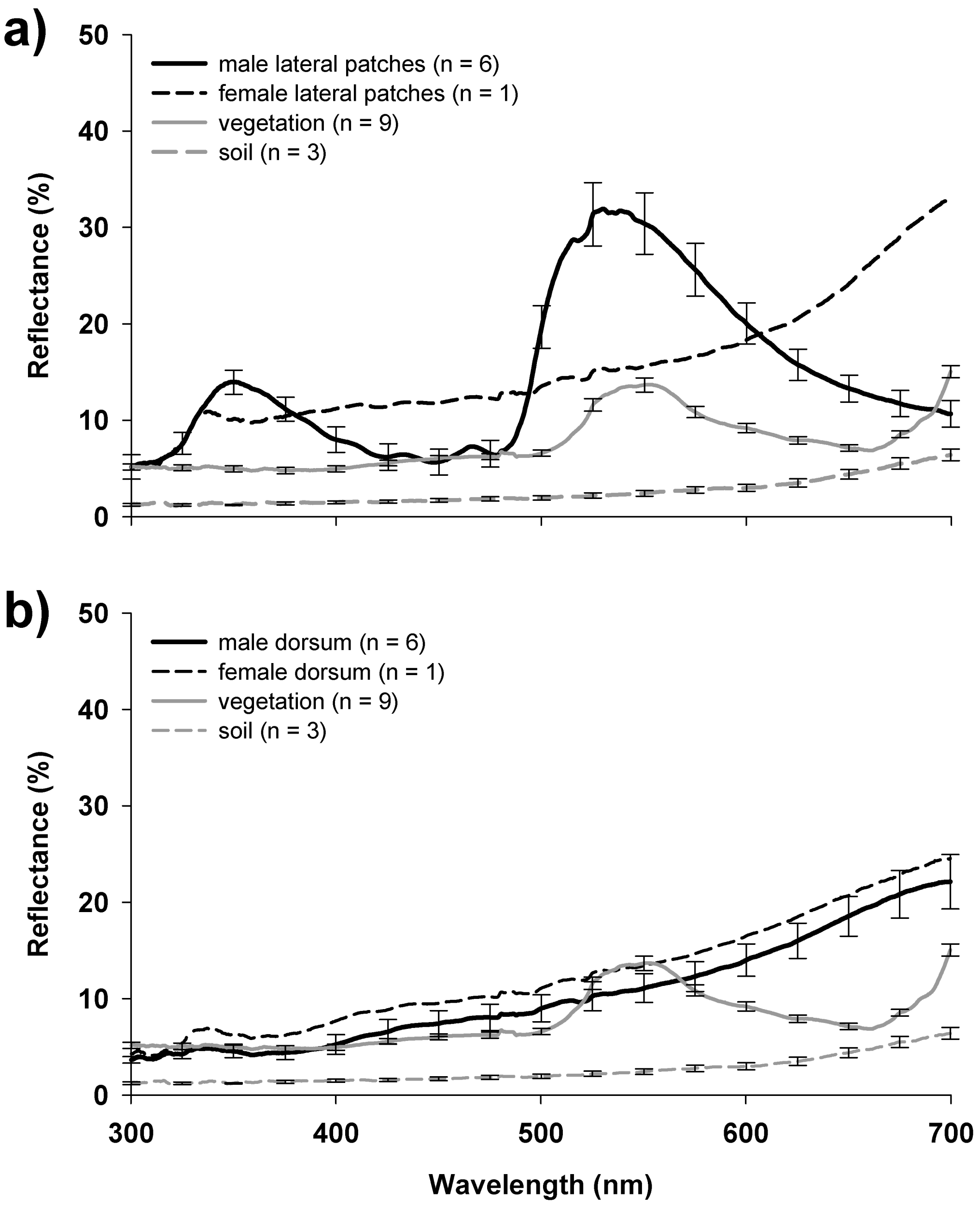

Figure 1. Reflectance spectra of lateral patches (a) and dorsum (b) of *L. agilis* and natural backgrounds (a, b). Vertical lines = error bars (±1 SEM).

2001; Fleishman and Persons, 2001; Leal and Fleishman, 2002; Stoehr and McGraw, 2001).

Chromatic cues may support sex and species discrimination, and male colouration is involved in sexual and agonistic interactions (reviewed in Cooper and Greenberg, 1992). A possible role in social signalling has also been suggested for the UV-reflecting colour patches found in many lizards (e.g. Molina-Borja et al., 2006; Stapley and Whiting, 2006; Whiting et al., 2006). UV reflectance makes male sand lizards more conspicuous to receivers with an ability to see UV because natural visual backgrounds absorb wavelengths below 400 nm (fig. 1). Thus, UV reflectance may act as an amplifier (Hasson, 1989) enhancing the visual perception of the size of the nuptial colour patches (Cummings et al., 2006; Lappin et al., 2006). The fact that the UV reflecting patches are located laterally and ventrally, and thus remain relatively concealed until revealed by display postures (e.g. through sagittal compression), is also consistent with a signalling function (Olsson, 1992).

However, the presence of UV-reflecting skin patches does not prove that sand lizards can see into the UV part of the spectrum or that UV colour patterns play a role in sand lizard social interactions. Swiezawska (1949) studied colour discrimination within the human-visible spectrum and found that sand lizards are most sensitive in the yellow-green region of the spectrum, which roughly corresponds to the body colouration of males, but no study to date has examined the ability of sand lizards to see into the UV. Nevertheless, the green patches of male sand lizards reflect very close to the absorption wavelength peak of UV receptors in lizards with known UV vision (Fleishman et al., 1993; Loew, 1994; Ellingson et al., 1995; Loew et al., 1996, 2002), and preliminary results using elecroretinography suggest that lacertids have a broad spectral sensitivity extending from UV (350 nm) to deep red (700 nm) (Palacios, Herrera, Desfilis and Font, unpublished data).

In contrast with the present study, previous studies of sand lizards from Sweden reported no UV reflectance of the male nuptial coloration. Reasons for the discrepant results of these studies are unclear. One obvious possibility is that the lack of UV reflectance in Swedish populations (or its presence in Pyrenean sand lizards) is the result of geographic colour variation. In fact, Swedish and Pyrenean populations are in opposite north-south ends of the distribution range of sand lizards. Moreover, Swedish populations are relict from the period of warmth that followed the last glacial episode (Gullberg et al., 1999), whereas Pyrenean sand lizard populations may be the result of a recent colonization (Llorente et al., 1997). Geographic variation in colour pattern is widespread in lizards (e.g. Baird et al., 1997; Kwiatkowski and Sullivan, 2002; Macedonia, 2001; Carretero, 2002; Thorpe, 2002; Thorpe and Stenson, 2003; Macedonia et al., 2003, 2004; Stuart-Fox et al., 2004; Rosenblum, 2005). However, there are few reports of geographic variation in UV colouration. Previously we found geographic variation in the UV reflectance of blue lateral patches of *Gallotia galloti* lizards from Tenerife (Font and Molina-Borja, 2004; Molina-Borja et al., 2006). Although spectrograms of Swedish sand lizards have not been published, the contrasting colouration of Swedish and Pyrenean populations of sand lizards represents, to our knowledge, the most extreme geographic UV colour variation known in any vertebrate.

The presence of UV reflectance in the nuptial patches of male sand lizards has strong implications for future studies of the evolution of conspicuous lizard colouration. It also reinforces the need to assess geographic variation in signalling systems, including cases in which the variation has components beyond direct human perception (i.e. below the 400 nm limit of human colour vision). As stressed several times recently (e.g. Cuthill and Bennett, 1993; Bennett et al., 1994; Thorpe, 2002), descriptions of colours or colour patterns that do not take into account the animals' perception may result in an incomplete assessment of visual signals and risk misinterpretation. Therefore, the potential visual sensitivity to UV wavelengths should be taken into account in future behavioural studies of UV-reflective lizards. Such elaborate UV colouration as has been found in sand lizards and in other lacertids suggests that lizards may use more complex visual signals than previously thought.

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