Intraspecific variation and detectability of iridescence in the dorsal coloration of a wall lizard

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Abstract (296 words)

Iridescence refers to the optical property of surfaces for which reflected wavelengths depend on viewing geometry. Although iridescence underlies some of the most striking animal colours, the sensory stimulation elicited by iridescent spectral shifts in relevant observers has seldom been explored. Wall lizards often show substantial intraspecific colour variation, which may influence the detectability of iridescence by varying the range of affected wavelengths. Here, we set out to study *Podarcis liolepis* dorsal coloration in two localities selected for their colour differences: the València Botanical Garden (EB), and La Murta Natural Park (LM). To determine the presence of angle-dependent reflectance, we measured dorsal coloration at three different configurations (0º, 60º, and 90º angles between incident light and observer location) in 87 lizards of either sex and used visual modelling to determine their detectability when viewed by conspecifics, raptors, and humans. Our results show that *P. liolepis* dorsal coloration varies chromatically with sex and locality, and also shows iridescence (i.e. reflectance peaks at shorter wavelengths with increasing viewing angle). Lizards from EB are brown dorsally, whereas most lizards from LM, especially males, show a green dorsal background coloration, reflectance peaking at shorter wavelengths in lizards from LM compared to lizards from EB. Angle-dependent shifts in peak location are similar across localities and sexes, yet increased overlap between the involved waveband and receiver cone sensitivities results in larger chromatic distance in lizards from LM (compared to EB) for every observer considered. In addition, *P. liolepis* dorsal iridescence may be more apparent to humans and raptors than to the lizards themselves. Our findings suggest that intraspecific colour variation may affect the detectability of iridescence consistently across observers, emphasizing the importance of using objective colour quantification and visual modelling methods when studying the ecological consequences of iridescence in nature.

Keywords

Iridescence, sexual dichromatism, intraspecific colour variation, spectrophotometry, visual modelling, reptile coloration, sensory ecology, wall lizards

Introduction

Iridescence refers to the optical property of a surface for which the spectral composition of reflected light changes with the angle between observer and illumination source (i.e. hereafter, viewing geometry or angle) (Stuart-Fox *et al.*, 2021; Ospina-Rozo, Roberts & Stuart-Fox, 2022). More precisely, while iridescence describes angle-dependent shifts in the chromatic component of spectral shape (e.g. pointedness and wavelength of peak reflectance), the term specularity is used to refer to any achromatic changes (i.e. angle-dependent mean reflectance) (Stuart-Fox *et al.*, 2021). Iridescence underlies some of the most striking animal colours and is a particularly challenging case for researchers, as its cellular basis, development, and adaptive significance are still poorly understood. Iridescence is produced by the physical interaction between light and nanometer-scale variation in the arrangement and refractive indices of biological structures in the integument (Onslow, 1923; Land, 1972; Doucet & Meadows, 2009). However, the mechanisms involved in the distinction between iridescent and non-iridescent structural colours remain elusive (Land, 1972; Meadows *et al.*, 2009; Seago *et al.*, 2009; Stuart-Fox *et al.*, 2021). The perception of objective shifts in spectral shape as subjective chromatic changes depends on the interplay between the range of affected wavelengths and features of the receiver visual system, and hence research on iridescence should clearly differentiate between the physical, sensory, and psychophysical aspects of the phenomenon (Ospina-Rozo *et al.*, 2022).

Iridescence is relatively common in invertebrates (e.g., crustaceans, Parker, 2000; molluscs, Mäthger *et al.*, 2009; insects, Miaoulis & Heilman, 1998; Seago *et al.*, 2009). However, iridescence in vertebrates is comparatively rare and taxonomically restricted, with most of the known cases concerning birds and fish (Denton, 1970; Lythgoe & Shand, 1989; Osorio & Ham, 2002; Prum, 2006). Iridescent colorations have also been described in a few species of lizards and snakes (Rohrlich & Porter, 1972; Morrison, 1995; reviewed in Doucet & Meadows, 2009). Reports of iridescence in the family Lacertidae (a widely distributed taxon comprising about 360 species) are limited to two species: the Iberian emerald lizard, *Lacerta schreibeiri* (Pérez i de Lanuza & Font, 2014a), and the common wall lizard, *Podarcis muralis* (Pérez i de Lanuza & Font, 2016). Given the diversity of body colorations found in lacertid lizards (Pérez i de Lanuza, Font & Monterde, 2013), the likelihood of underreported iridescence within this group is considerable. Furthermore, wall lizards are well-known for their substantial intraspecific variation regarding coloration (Brock, McTavish & Edwards, 2020; Miñano *et al.*, 2021; Storniolo *et al.*, 2021), which may have potential implications for the detectability of iridescence by varying the range of affected wavelengths.

Podarcis liolepis (Boulenger, 1905; formerly *P. hispanica*) is a small lacertid lizard found in relatively dry Mediterranean areas with rocky substrates throughout the eastern Iberian Peninsula and the Mediterranean coast of France (Renoult *et al.*, 2009). Dorsal coloration of *P. liolepis* is variable across its distribution range (Renoult *et al.*, 2010), although this geographical chromatic variability remains understudied. In most of its distribution range, dorsal coloration consists of black spots or stripes over a brownish background, although the black patterning is more nuanced or even absent in some places, as in the city of Valencia (i.e., *concolor* phenotype; Salvador, 2014). In contrast, in some areas, especially in the southern part of the species' distribution range, lizards often exhibit a green background dorsal coloration, especially males. Although the taxonomic status of the species originating from the former *P. hispanicus* complex is still under scrutiny, it is unlikely that this chromatic variation may result from hybridization between different species (Renoult *et al.*, 2009, 2010; Kaliontzopoulou *et al.*, 2011; Salvador & Carretero, 2014). Our aims here are: 1) to describe the spectral differences in the dorsal coloration of *P. liolepis* lizards between two localities and evaluate their detectability to a lizard observer, 2) to examine the putative existence of angle-dependent reflectance properties (i.e. iridescence) in *P. liolepis* dorsal coloration, and 3) to explore the consequences of interpopulation colour variation on the detectability of iridescence by modelling the sensory response of a range of selected observers (i.e. lizards, raptors, humans). Our focus for this last objective is on exploring how intraspecific colour variation may consistently affect the detectability of iridescence in some populations. Therefore we modelled the sensory response of lizards and raptors (two ecologically relevant observers possessing four different types of cones in their retina, yet differing in their peak wavelength sensitivities), as well as humans (i.e. possessing three types of cones and responsible for descriptions of intraspecific colour variation in field guides and scientific studies).

Materials and methods

Subjects

In May 2021 we captured by noosing 38 adult *P. liolepis* (21 females and 17 males) in the Botanical Garden of the University of Valencia (EB, 39° 28' N, 0° 23' W; Valencia), and 49 adults (17 females and 32 males) in la Murta i la Casella Natural Park (LM, 39° 07' N, 0° 21' W; Alzira). Both localities are separated by approximately 35 km. Whereas all the lizards from EB are brown dorsally, most lizards from LM, especially males, show a green dorsal background coloration (Fig. 1). To study dorsal coloration and its angle-dependent spectral properties, we measured dorsal reflectance in each lizard at three different viewing geometries, determined by the angle between the incident light and the measuring probe (0°, 60°, and 90°). For the 0º setup we used a single probe combining 6 emissive fibres and a recording fibre held perpendicularly to the lizard's skin surface (Font, Pérez i de Lanuza, & Sampedro, 2009). For the 60° and 90° setups, we used two independent emissive and recording probes mounted on a purpose-built goniometer that allows probes to be rotated to a precise angular position (for a detailed description see Pérez i de Lanuza & Font, 2014b). For 60° and 90° we took two subsets of measurements to control for the lizard orientation in relation to the emissive and recording probes. Thus, we first placed the emissive probe facing tailward (i.e., head to tail) and then we placed it headward (i.e., tail to head) with respect to the lizard. Spectrophotometric measurements were obtained using a USB-2000 portable diode-array spectrometer and a PX-2 xenon strobe light source (Ocean Optics, Dunedin, FL). We attached an entomological pin to the side of the probe (nylon head down), which allowed us to maintain a constant distance of 3 mm between the tip of the probe and the target surface. We set the integration time to 70 ms, the number of readings per average to 20, and boxcar width to 10 (Font, Pérez i de Lanuza & Sampedro, 2009b; Badiane *et al.*, 2017). For analyses, we restricted the reflectance spectra to the 300-700 nm range to encompass the visual sensitivity of lacertid lizards (Pérez i de Lanuza & Font, 2014b; Martin *et al.*, 2015).

Statistics

Spectral data were analyzed in R v.4.0.3 (R Development Core Team, 2017) using the package *pavo 2* (Maia *et al.*, 2019). Spectra were smoothed (span = 0.2) and normalized by making the reflectance at all wavelengths proportional to the minimum reflectance. The tilt in the incident beam of light when reflectance is measured at growing angles between the emissive and receptive probes decreases the amount of reflected light that enters the receptive probe, thus producing an artefactual negative relationship between intensity and viewing angle. Since this intensity reduction is especially noticeable in the UV range (i.e. 300-400 nm), values below 400 nm were homogenized by equating them to the value at 400 nm. This homogenization cannot alter substantially the analyses because the dorsal coloration has no or very little UV reflection in this species (Fig. 1 in Pérez De Lanuza & Font, 2016). Thus, for each spectrum we calculated four standard variables describing colour: total reflectance (i.e., luminance; just for the 0° setup), peak location, and two measures of chroma (Endler, 1990; Kemp *et al.*, 2015). We calculated total reflectance (Qt) as the sum of the reflectance across the visible range of lizards (i.e., $R_{300-700}$) and peak location as the wavelength of maximum reflectance (λ_{max}). To study purity of green and brown dorsal colorations we calculated green chroma (GC) as the relative reflectance in the middle-wavelength range (i.e., summing the reflectance in the 490-570 nm range and dividing it by total reflectance; $R_{490-570}/R_{300-700}$), and brown chroma (BC) as the relative reflectance in the long-wavelength range (i.e., $R_{571-700}/R_{300-700}$) (Endler, 1990; Pérez i de Lanuza, Carazo & Font, 2014).

Visual models

We built visual models using the Vorobyev and Osorio receptor noise model (Vorobyev & Osorio, 1998) implemented in *pavo 2* to assess colour discrimination in terms of chromatic and achromatic distances, in order to test whether lizards are able to sense the differences between the dorsal colorations of 1) conspecifics when observed with different viewing geometries, 2) males and females from the same population, 3) lizards from the two populations, and (4) both subsets of measurements taken with a viewing angle of 60° and 90° (i.e., tailward and headward). As we did not find any difference between the two subsets of measurements (tailward and headward) taken with a viewing angle of 60° and 90° (adjusted $p > 0.05$), we pooled both spectra subsets for further analyses. Since detailed information on the visual system of *P. liolepis* is not available, we used cone sensitivities (UVS:SWS:MSW:LSW, 367:456:497:562 nm) and cone ratios (1:1:1:4) of its close relative *P. muralis* (Martin *et al.*, 2015). In the absence of behavioral estimates of the correct

Weber fraction for colour discrimination in lizards, we set the Weber fraction to 0.1 (as it has been estimated for bird long-wavelength sensitive class cones; Vorobyev and Osorio, 1998), and a standard daylight "D65" irradiance spectrum, as implemented in *pavo*. For the bird predator model we used the violet sensitive (VS)-averaged cone sensitivity included in *pavo*, as it approximates the visual system of the Falconiformes (Ödeen & Håstad, 2003; Stoddard & Prum, 2008), which are the most typical avian predators of *P. liolepis* (Martin & López, 1990; Salvador, 2014) and also possess four different types of cones in their retina (Cronin & Bok, 2016). We used a cone ratio of 1:2:2:4 (UVS:SWS:MSW:LSW) and a Weber fraction of 0.1 (Vorobyev *et al.*, 1998). For the human models we used the LMS cone sensitivities (Stockman & Sharpe, 2000), cone ratios 0.057:0.314:0.629 (SWS:LWS1:LWS2; Hofer *et al.*, 2005), and two different Weber fractions: 0.02 (as has been estimated for the human LWS cone class (Wyszecki & Stiles, 2000) but also 0.1 for the sake of comparison with birds and lizards.

Contrasts between pairs of colours were measured in units of just noticeable differences (JND), where one JND is the threshold of discrimination between two colours under good illumination conditions (Vorobyev & Osorio, 1998). However, as JND values between one and three could mean that two colours are barely discriminated, we also evaluated our results using a more conservative discrimination threshold of 3 JNDs (Siddiqi *et al.*, 2004; Santiago *et al.*, 2020). We used a distance-based PERMANOVA (Anderson, 2014) to test for statistical differences between dorsal colorations with respect to sex, locality, and viewing angle. To do so, we ran a PERMANOVA procedure on the chromatic and achromatic contrasts using the *pairwise.adonis* function from the *pairwiseAdonis* R package (Martinez Arbizu, 2017), a modified version of the *adonis* function from the *vegan* R package (Oksanen *et al.*, 2016) allowing for multilevel pairwise comparisons taking into account repeated measures. For analyses on reflectance spectra measured at 60° and 90°, we only ran the PERMANOVA procedure on chromatic contrasts, since the procedure to measure reflectance with increasing angle geometry generates spurious luminance changes. When comparing reflectance spectra from the same individual taken at different angles, we accommodated the repeated measure nature of the data in the PERMANOVA by blocking contrasts at the within-individual level. Finally, as significance thresholds do not necessarily match the theoretical discriminability threshold of one JND above which colours can be said to be distinguishable, we used a bootstrap procedure to generate confidence intervals for the mean colour distance between the different colours. We used the *bootcoldist* function from the *pavo* package on the visual model described above with 1000 replicates and a 0.95 level for confidence intervals.

Results

Compared to lizards from EB, lizards from LM show their peak reflectance displaced towards shorter wavelengths, hence showing an overall greener coloration to the human eye (Table 1; Figures 1 and 2A). Dorsal coloration differs statistically in both chromatic and achromatic contrasts between both localities for every same-sex comparison except for females measured with the 0° setup (Table 2). At a 0º viewing angle, chromatic differences between males from different localities are more apparent to conspecifics than differences between females from different localities (Fig. 2B). Comparing sexes within each locality, we found statistically significant differences only in LM with the 0º setup (Table 2). Accordingly, chromatic distances between sexes are larger in LM than in EB (Fig. 2B).

Males and females from both localities show angle-dependent spectral shifts, with reflectance peaking at shorter wavelengths when increasing the angle between incident light and observer viewpoint (Table 1; Video V1). Thus, the dorsal coloration of *P. liolepis* is iridescent, appearing greener with wider viewing angles (i.e. 90º). Angle-dependent peak variation differs in range and magnitude between localities, and between sexes in LM (i.e. females showing more pronounced changes than males) but not in EB (Fig. 3).

For every potential receiver examined, chromatic distances between spectra measured at different viewing angles (measured in JNDs) were larger in LM than in EB. According to our visual models, angle-dependent chromatic changes are more noticeable to conspecifics in LM than in EB (Fig. 4). In lizards from LM, angle-dependent changes are significant for every pair of angles compared (Table 3) and likely chromatically discriminable to conspecifics between 0º-90º (JND ± CI_{95} > 3; Fig. 4). By contrast, angle-dependent changes in lizards from EB are less noticeable to conspecifics and perhaps not sensed as chromatically distinct (JND ± CI_{95} < 3; Table 3; Fig. 4). To putative avian predators and humans, angle-dependent changes are significant for every pair of angles compared (Table 3), but iridescent shifts are probably only sensed as categorically distinct colours in LM (JND ± CI_{95} > 3; Fig. 4). Chromatic distances between paired spectra measured at different viewing angles are larger in raptors and humans with respect to lizards (e.g. mean 0º-90º JNDs in females from la Murta: lizards = 4.09; humans = 6.13; birds = 7.91).

Discussion

Our results show that the dorsal coloration of *Podarcis liolepis* varies with locality and sex, but also with viewing geometry. Dorsal reflectance peaks at shorter wavelengths in lizards from LM than in lizards from EB. Lizards from LM are sexually dichromatic, with males peaking at shorter wavelengths than females and therefore appearing greener to the human eye, while there are no sex differences in dorsal background coloration in EB, where both males and females look brownish (Fig. 1). The difference between the sexes in LM is independent of viewing angle and large enough to be chromatically discriminated by conspecifics (Fig. 2B). We also found that the dorsal coloration of *P. liolepis* is iridescent, peaking at shorter wavelengths with increasing angle between incident light and observer viewpoint (Table 2 and Fig. 3). The iridescent properties of *P. liolepis*' dorsal coloration are similar to those reported in the closely related *P. muralis* (Pérez i de Lanuza & Font, 2016). Although iridescence is present in *P. liolepis* from both studied localities, for all receivers examined similar angle-dependent spectral shifts are more apparent in LM lizards than in EB lizards, at least based on visual models and their corresponding assumptions. This results from the existence of a sensory dimension to iridescence by which spectral shift is a poor predictor of stimulation in the observer (Fig. 3 and 4).

Sensory stimulation increases when the range of variation encompasses a lager fraction of the overlap in sensitivity between more than one type of cone in the retina of the receiver (Kelber, Vorobyev & Osorio, 2003; Kelber & Osorio, 2010). For instance, the spectral shift between the 0º and 90º setups is as large in females from EB as in females from LM (~75 nm). Nonetheless, this spectral shift results in a relatively smaller chromatic distance to conspecifics in EB because the range of variation falls within 663 and 584 nm, while in LM falls within the 626-550 nm range (matching a larger fraction of the overlap between the MW and the LW *Podarcis* cones; Pérez i de Lanuza & Font, 2014b; Martin *et al.*, 2015). Likewise, the spectral shift between 0º and 90º setups is larger in males from EB than in males from LM (EB ~ 82 nm; LM ~ 57 nm), but the chromatic distances to conspecifics are considerably larger in LM than in EB (EB = 1.83 JND; LM = 4.09 JND). Increased overlap between angle-dependent spectral changes and cone sensitivities is likely responsible also for our results considering other potential receivers, which according to visual modelling may perceive iridescent changes in *P. liolepis* dorsal coloration even better than the lizards themselves (Fig. 4; Fig. S1). This is particularly evident in our avian vision models, which estimate larger chromatic distances compared to lizards (and slightly larger than humans) despite using the same Weber fraction of 0.1. Research on humans demonstrates that the relationship between differential sensory stimulation and perceptual distance is intricate and nonlinear (Vienot, 2002; Witzel, 2019; Luo, 2020). Therefore, results from visual modelling should be interpreted as providing information on the sensory input available to the colour-processing neural channels in a given species, and not as definitive proof of colour discrimination (Osorio & Vorobyev, 2008; Baden & Osorio, 2019). Although iridescence being more pronounced in LM than in EB aligns with our own visual impression, we should be cautions with results coming from visual models because, unfortunately, some crucial information is not available. For example, in the coral reef fish *Rhinecanthus aculeatus*, the relationship between behaviourally-determined detectability of colour stimuli and model-based chromatic contrast against background varied across the colour space and followed a sigmoidal function (Cheney *et al.*, 2019; Santiago *et al.*, 2020). Conducting behavioural experiments in *Podarcis* lizards can validate results from visual models by observing how lizards process and discriminate light within a specific waveband of interest, bridging the gap between sensory input estimations and repeatable demonstrations of colour discrimination ability (Kelber *et al.*, 2003; Pérez i de Lanuza *et al.*, 2018).

Intraspecific variation involving green and brown background dorsal colorations has been described in many *Podarcis* species (e.g., *P. muralis, P. vaucheri, P. pityusensis, P. lilfordi*, *P. melisellensis, P. gaigae, P. filfolensis, P. cretensis, P. peloponnesiacus, P. raffonei, P. tauricus, P. virescens, P. wagleriana;* Gorman *et al.*, 1975; Bauwens & Castilla, 1998; Podnar, Mayer, & Tvrtković, 2004; Salvador, 2014; While *et al.*, 2015; Faria, 2019; Cirer, 2020). In addition, sexual dichromatism along a green-brown axis is also common in *Podarcis*, with males often showing green-biased dorsal coloration and females more frequently appearing brown (e.g., *P. bocagei*, *P. siculus*, *P. melisellensis, P. pityusensis;* Galán, 2008; Salvador, 2014; Storniolo *et al.*, 2021). For many of these species, colour variation has been characterized in the literature according to human vision (sometimes even from preserved museum specimens), disregarding other chromatic phenomena such as iridescence. Our results suggest the need of reevaluating colour variation in these species considering the visual system of lizards and other (potentially) relevant observers, as well as viewing geometry, since iridescence can play a crucial role in the detection of colour for all receivers, including humans. In turn, colour descriptions coming from field guides, photography-based sampling, or citizen science projects may need to be updated with new information on the potential angle-dependent properties of animal coloration.

Several different hypotheses have been put forward regarding the functional consequences of animal iridescence in contexts such as communication, predator avoidance, orientation, thermoregulation, photoprotection, friction reduction, water repellence, and strengthening (Doucet & Meadows, 2009; Pérez i de Lanuza & Font, 2014a). Traits involved in intraspecific communication are usually tailored to be more noticeable to conspecifics than to unintended observers (Cronin *et al.*, 2000; Marshall & Stevens, 2014). For instance, iridescence in the seasonal blue coloration of male heads in *L. schreiberi* increases its conspicuousness against a set of natural backgrounds when observed from wider viewing angles (more commonly experienced by conspecifics) rather than the acute angles typical of avian predators. This suggests an adaptive function related to signal detection and predator avoidance (Pérez i de Lanuza & Font, 2014a). A similar explanation is unlikely to apply to the dorsal iridescence of *Podarcis* lizards, which adopt varied orientations in relation to observers and sunlight while perching on vertical rock walls so that both conspecifics and predators are unlikely to differ significantly in their average viewing angles when observing the lizards' dorsal surface. Regardless of its consequences for background-matching and the lizards ability to exploit light directionality, iridescence may still play a role in predator avoidance if a more changeable appearance may startle potential predators, thwart their ability to judge distances, or hinder the acquisition of a search image (Doucet & Meadows, 2009; Kjernsmo *et al.*, 2020). Our finding that iridescence-induced changes in colour sensation may be more apparent to other potential observers than to lizards themselves underscores the worth of furtherly exploring the impact of lacertid dorsal iridescence on predator avoidance. However, we should also consider the possibility that iridescence may not confer any selective advantage, and simply constitute a non-adaptive correlate of other traits under selection (Doucet & Meadows, 2009). For instance, iridescent properties have also been described in the ventral surface of *P. liolepis* (Pérez i de Lanuza & Font, 2016), an observation that is difficult to interpret from a purely adaptationist point of view (e.g., the ventral surface of lacertid lizards is rarely exposed to predators or conspecifics).

Altogether these findings imply that iridescence might be underreported in reptiles, introducing an additional dimension of variation to the already remarkable colour diversity of

Podarcis lizards. Moreover, our research highlights that intraspecific colour variation affects the detectability of iridescence consistently across observers, which may have implications regarding our knowledge on animal colour diversity by raising awareness on the possible existence of unnoticed angle-dependent properties in other taxa. Overall, our research demonstrates that objective colour quantification at different viewing geometries and the use of visual modelling to evaluate detectability by relevant observers is crucial to understand the evolutionary causes and consequences of iridescence in nature.

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Author contributions

Conceptualization: F.C., J.A., G.P.L., E.F.; Investigation: F.C., J.A., G.P.L., E.F.; Statistical analysis: F.C.; J.A.; Writing – original draft: F.C.; J.A.; Writing – review & editing: F.C., J.A., G.P.L., E.F.

Conflict of interests

The authors declare no conflict of interests.

Data accessibility statement

The dataset used for this study can be obtained at 10.5281/zenodo.10795952.

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Tables

Table 1. Mean ± SEM total reflectance (Qt), green chroma (GC), brown chroma (BC), and peak location (λ_{max}) of *P. liolepis* by locality (LM = la Murta, EB = el Botànic), sex (m = males, f = females), and viewing angle. Total reflectance of 60° and 90° setups are not reported because of the artefactual negative relationship between intensity and viewing angle.

Locality	**Sex**	**N**	**Angle**	**Qt**	**GC**	**BC**	**λ_{max} (nm)**
LM	m	32	0º	1262 ± 61	0.33 ± 0.02	0.60 ± 0.02	592.7 ± 4.4
			60°	-	0.45 ± 0.01	0.47 ± 0.01	558.2 ± 2.8
			90°	-	0.50 ± 0.01	0.39 ± 0.01	535.9 ± 1.5
	f	17	0º	1007 ± 46	0.23 ± 0.01	0.70 ± 0.01	625.6 ± 3.3
			60°	-	0.34 ± 0.02	0.60 ± 0.02	586.0 ± 3.4
			90°	-	0.46 ± 0.02	0.43 ± 0.02	550.4 ± 3.1
EB	m	17	0º	1653 ± 97	0.19 ± 0.00	0.75 ± 0.01	662.2 ± 3.5
			60°	-	0.25 ± 0.01	0.67 ± 0.01	617.1 ± 3.8
			90°	-	0.35 ± 0.01	0.55 ± 0.01	579.8 ± 3.6
	f	21	0º	1516 ± 58	0.18 ± 0.00	0.76 ± 0.01	662.6 ± 3.2
			60°	-	0.25 ± 0.01	0.68 ± 0.01	621.7 ± 3.6
			90°	-	0.56 ± 0.01	0.26 ± 0.01	583.9 ± 3.9

Table 2. Pairwise comparisons between the dorsal coloration of same-sex *P. liolepis* lizards from la Murta (LM) and el Botànic (EB), and between males and females from both localities. Contrasts were calculated using a distance-based PERMANOVA on the chromatic and achromatic distances obtained by modelling the vision of conspecifics (Weber fraction = 0.1) at three different viewing angles (i.e. 0º, 60º, 90º). Significant contrasts ($p < 0.05$) are indicated in bold. F and R^2 represent pseudo F-statistics and effect size estimate, respectively. Adj. p represents adjusted p values (Bonferroni correction).

Pairwise	**Angle**	**Chromatic contrast ΔS**				**Achromatic contrast ΔL**			
		F	R^2	*p*	adj. *p*	*F*	R^2	*p*	adj. *p*
LM (♀ - ♂)	0°	5.96	0.11	0.004	**0.004**	6.10	0.11	0.009	**0.009**
	60°	2.1	0.04	0.120	0.120	-	-	-	**-**
	90°	0.45	0.01	0.682	0.682	-	-	-	**-**
EB (♀ - ♂)	0°	2.29	0.06	0.127	0.127	0.93	0.03	0.379	0.379
	60°	0.24	0.01	0.840	0.840	-	-	-	-
	90°	2.78	0.07	0.076	0.076	-	-	-	-
LM - EB (♂ - ♂)	0°	10.5	0.18	0.001	**0.001**	5.32	0.10	0.009	**0.009**
	60°	9.22	0.16	0.001	**0.001**	-	-	-	**-**
	90°	10.2	0.18	0.001	**0.001**	-	-	-	**-**
LM - EB (♀ –♀)	0°	1.02	0.03	0.373	0.373	26.6	0.42	0.001	**0.001**
	60°	7.03	0.16	0.002	**0.002**	-	-	-	**-**
	90°	3.06	0.08	0.045	**0.045**	-	-	-	**-**

Table 3. Pairwise comparison among different viewing angles of *P. liolepis* dorsal coloration (i.e. 0º, 60º, 90º), by sex (m = males, f = females) and locality (La Murta, El Botànic). Contrasts were calculated using a distance-based PERMANOVA on the chromatic distances obtained when modelling the vision of lizards, raptors, and humans (Weber fraction = 0.1). Significant contrasts ($p < 0.05$) are indicated in bold. *F* and R^2 represent pseudo *F*-statistics and effect size estimate, respectively. Adj. *p* represents adjusted *p* values (Bonferroni correction).

Receptor	**Locality**	**Sex**	**Pairwise**	**Chromatic contrast ΔS**			
				F	R^2	*p*	adj. *p*
Lizard	La Murta	m	0° - 60°	11.03	0.15	0.001	**0.001**
			60° - 90°	11.77	0.16	0.001	**0.001**
			0° - 90°	38.717	0.38	0.001	**0.001**
		f	0° - 60°	6.08	0.16	0.028	**0.020**
			60° - 90°	5.73	0.15	0.001	**0.001**
			0° - 90°	17.85	0.36	0.001	**0.001**
	El Botànic	m	0° - 60°	2.67	0.08	0.041	**0.035**
			60° - 90°	5.14	0.14	0.008	**0.004**
			0° - 90°	5.04	0.14	0.001	**0.001**
		f	0° - 60°	1.87	0.04	0.122	0.101
			60° - 90°	2.33	0.06	0.072	0.067
			0° - 90°	6.64	0.14	0.002	**0.002**
Raptor	La Murta	m	0° - 60°	29.45	0.32	0.001	**0.001**
			60° - 90°	26.0	0.30	0.001	**0.001**
			0° - 90°	99.46	0.62	0.001	**0.001**
		f	0° - 60°	13.69	0.30	0.001	**0.001**
			60° - 90°	30.52	0.49	0.001	**0.001**
			0° - 90°	63.34	0.66	0.001	**0.001**
	El Botànic	m	0° - 60°	8.39	0.21	0.001	**0.001**
			60° - 90°	35.54	0.53	0.001	**0.001**
			0° - 90°	51.24	0.62	0.001	**0.001**
		f	0° - 60°	9.51	0.19	0.001	**0.001**
			60° - 90°	35.0	0.47	0.001	**0.001**
			0° - 90°	50.25	0.56	0.001	**0.001**
Human	La Murta	m	0° - 60°	13.09	0.17	0.001	**0.001**
			60° - 90°	25.31	0.29	0.001	**0.001**
			0° - 90°	64.80	0.51	0.001	**0.001**
		f	0° - 60°	7.21	0.18	0.001	**0.001**
			60° - 90°	24.06	0.43	0.001	**0.001**
			0° - 90°	29.65	0.48	0.001	**0.001**
	El Botànic	m	0° - 60°	3.13	0.09	0.003	**0.005**
			60° - 90°	20.08	0.39	0.001	**0.001**
			0° - 90°	27.40	0.46	0.001	**0.001**
		f	0° - 60°	3.65	0.08	0.001	**0.002**
			60° - 90°	16.98	0.30	0.001	**0.001**
			0° - 90°	24.97	0.38	0.001	**0.001**

Figures

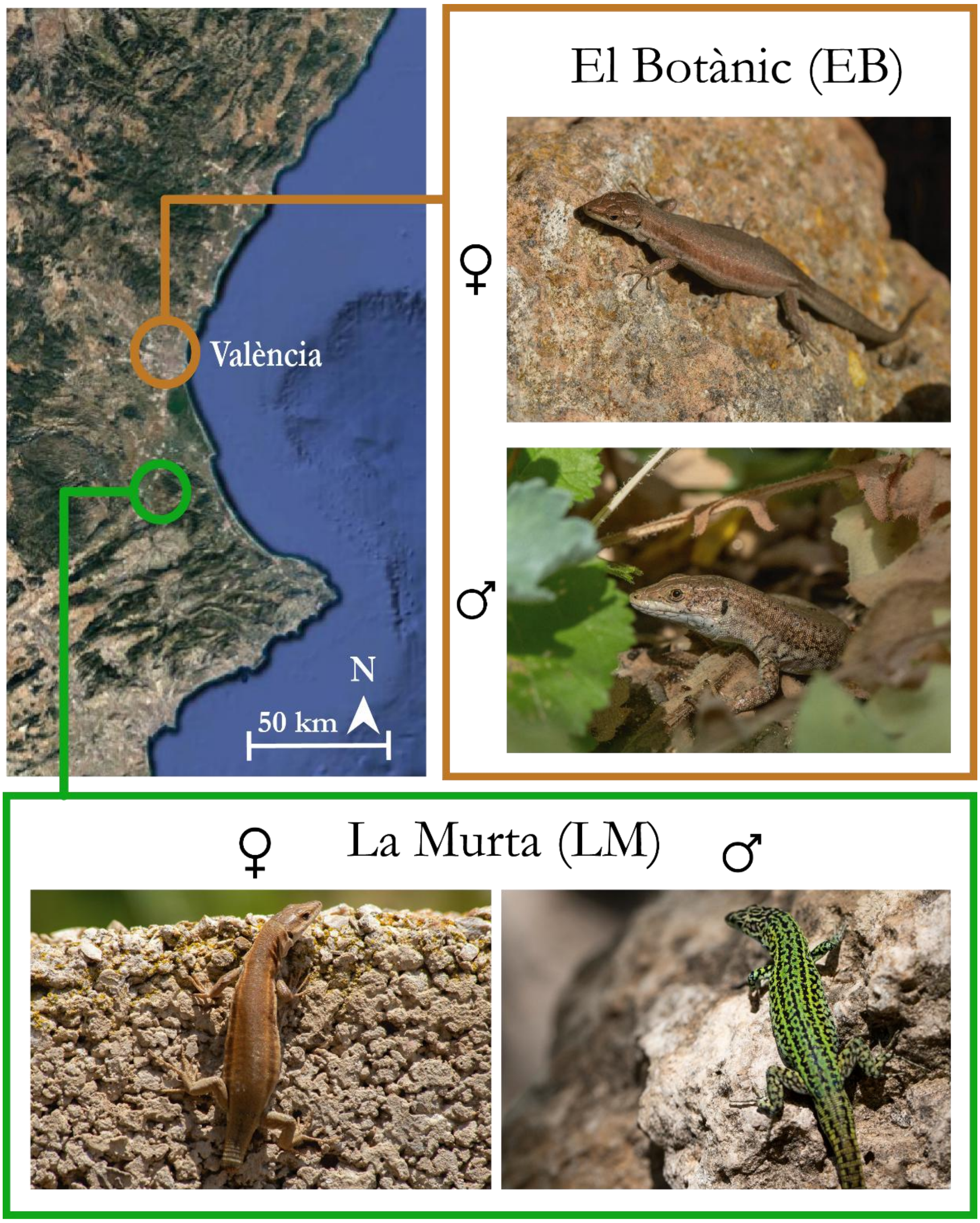

Figure 1. Satellite image of the Mediterranean coast of Valencia marking the location of the two sampling localities: EB (Botanical garden within the city of Valencia) and LM (Alzira, 35 km south of Valencia). On the right and below, photographs of females and males from both localities.

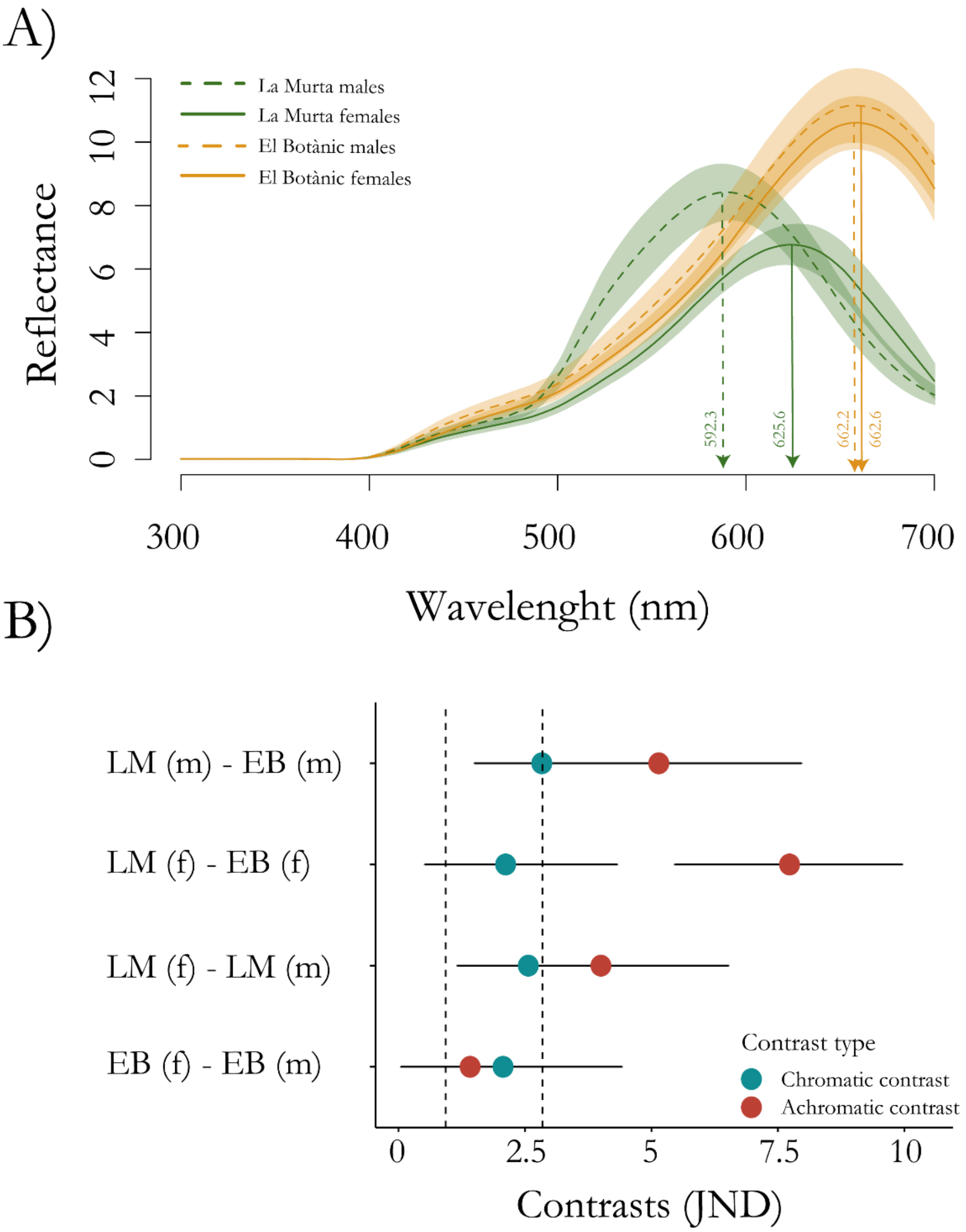

Figure 2. Variation of *P. liolepis* dorsal coloration by sex (m = males, f = females) and locality (La Murta, El Botànic). A) Mean reflectance spectra normalized by the minimum (line) and standard error (shaded area) of *P. liolepis* dorsal coloration classified by sex and locality (measurements taken with a viewing angle of 0°). Lizards from LM present their peak reflectance (arrows) at lower wavelengths than lizards from EB. In addition, males and females differ in their dorsal coloration at LM, but not at EB. B) Mean and 95% confidence intervals of the chromatic and achromatic distances between the dorsal coloration of same-sex lizards from both localities, and between sexes from the same locality (measured at 0º). JND stands for “Just Noticeable Differences”. Two dashed lines at 1 and 3 JNDs represent two theoretical discriminability thresholds. If the confidence interval of a point includes a discriminability threshold, the two colours are not distinguishable at this threshold according to receiver cone sensitivities and relative abundance.

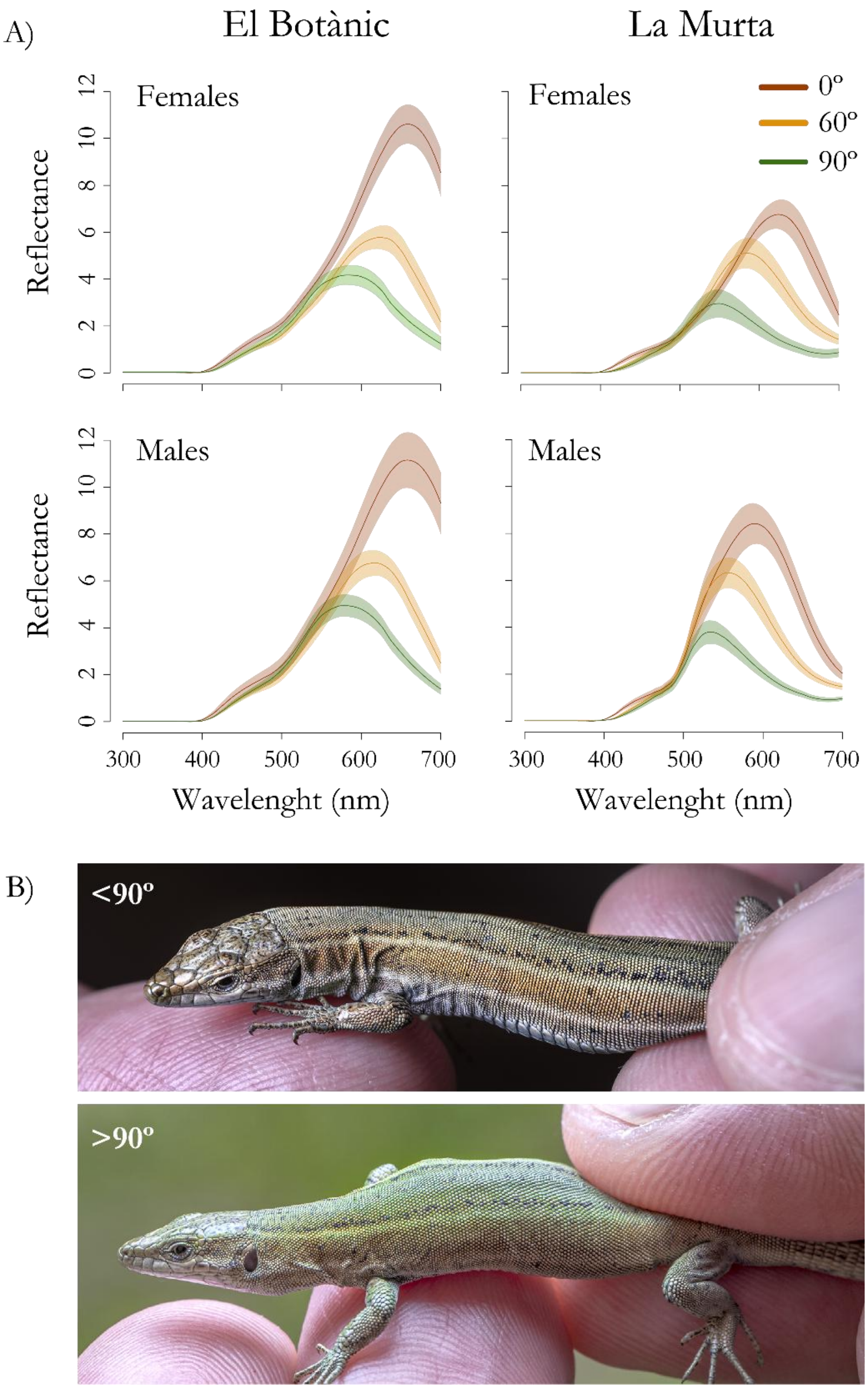

Figure 3. Chromatic variation of the dorsal coloration of *P. liolepis* according to viewing angle, sex, and locality. A) Mean normalized reflectance spectra (line) and 95% CI (shaded area) of *P. liolepis* dorsal coloration measured at a viewing angle of 0°, 60°, and 90°. Peak reflectance (arrows) for 0º and 90º is indicated, as well as 0º-90º spectral shift (double arrows). B) Photographs of the same female from LM with different viewing geometries. In the picture above, the sun is located behind the camera and thus the angle between incident light and observer viewpoint is narrower than 90º. In the picture below, the sun is located in front of the camera and therefore the viewing angle is wider than 90º.

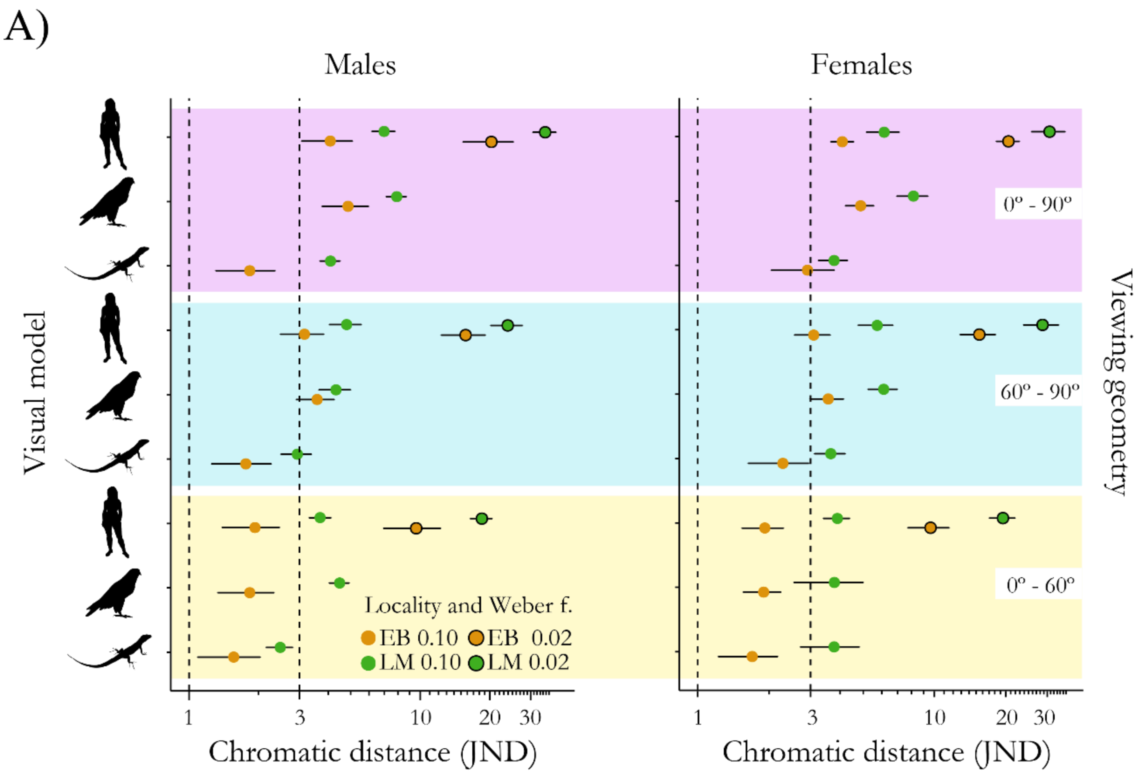

Figure 4. A) Mean and 95% confidence intervals of the within-individual chromatic distances between the dorsal coloration of *P. liolepis* lizards by sex and locality when viewed with three different viewing geometries (i.e. 0º, 60º, 90º), and according to the visual system of humans, VS-raptors, and wall lizards (Weber fraction = 0.10). For humans, we also show distances estimated with a Weber fraction of 0.02 (Wyszecki & Stiles, 2000). JND stands for Just Noticeable Differences. Two vertical dashed lines at 1 and 3 JNDs represent two theoretical discriminability thresholds. For more details on the correspondence between affected waveband and receiver cone sensitivities (underlying these results) see **Fig. S1** in the Supplementary Material.

Figure legends

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Appendix S1: Supplementary Material

Intraspecific variation in dorsal coloration and detectability of iridescence in a wall lizard

Tables

Table S1. Variation in percent reflectance between paired measurements of the same lizard obtained with different lizard orientations (head-to-tail and tail-to-head), presented by locality and viewing angle (EB = el Botànic; LM = la Murta).

	Mean reflectance [CI_{95}]	
	Locality	
Angle	**EB**	**LM**
60º	5.46 [5.42; 5.50]	4.78 [4.75; 4.81]
90º	4.69 [4.66; 4.72]	3.78 [3.76; 3.80]
	Mean standard deviation [CI_{95}]	
	Locality	
Angle	**EB**	**LM**
60º	0.65 [0.65; 0.66]	0.81 [0.80; 0.82]
90º	0.63 [0.62; 0.64]	0.68 [0.68; 0.69]
	Mean coefficient of variation [CI_{95}]	
	Locality	
Angle	**EB**	**LM**
60º	13.41% [13.23; 0.13.58]	17.89% [17.68; 18.09]
90º	14.71% [14.50; 14.92]	19.52% [19.26; 19.78]
	Mean difference in reflectance [CI_{95}]	
	Locality	
Angle	**EB**	**LM**
60º	0.12 [-0.02; 0.26]	0.07 [-0.10; 0.25]
90º	-0.04 [-0.18; 0.10]	0.11 [0.00; 0.23]

Table S2. Pairwise Tukey contrasts for differences in λ_{max} (wavelength of peak reflectance; nm) among viewing angles, divided by sex and locality (locality: EB = el Botànic; LM = la Murta; sex: F = Female; M = Male).

Locality	**Sex**	**Contrast**	**Estimate**	**SE**	**df**	***t* ratio**	***P-value***
EB	M	a0 - a60	45.1	2.84	46	15.88	<.0001
		a0 - a90	82.5	2.84	46	29.09	<.0001
		a60 - a90	37.5	2.84	46	13.21	<.0001
EB	F	a0 - a60	40.9	2.58	58	15.83	<.0001
		a0 - a90	78.8	2.58	58	30.50	<.0001
		a60 - a90	37.9	2.58	58	14.67	<.0001
LM	M	a0 - a60	34.5	2.57	91	13.423	<.0001
		a0 - a90	56.8	2.57	91	22.124	<.0001
		a60 - a90	22.3	2.57	91	8.70	<.0001
LM	F	a0 - a60	39.6	2.11	46	18.802	<.0001
		a0 - a90	75.2	2.11	46	35.68	<.0001
		a60 - a90	35.6	2.11	46	16.88	<.0001

Table S3. Pairwise contrasts for differences in λ_{max} (wavelength of peak reflectance; nm) across combinations of locality and viewing angles in same-sex *P. liolepis* lizards (*N* = 87). Contrasts are ordered in descending order according to the estimated difference. EB = el Botànic, LM = la Murta.

Females (*N = 38*)

Contrast	**Estimate**	**SE**	**df**	***t* ratio**	***P-value***
0º EB - 90º LM	112.57	4.7	108	23.93	<.0001
0º EB - 90º EB	77.21	1.72	108	44.793	<.0001
0º LM - 90º LM	77.21	1.72	108	44.793	<.0001
0º EB - 60º LM	75.7	4.7	108	16.093	<.0001
60º EB - 90º LM	72.23	4.7	108	15.355	<.0001
0º LM - 90º EB	41.85	4.7	108	8.896	<.0001
0º EB - 60º EB	40.34	1.72	108	23.404	<.0001
0º LM - 60º LM	40.34	1.72	108	23.404	<.0001
60º EB - 90º EB	36.87	1.72	108	21.389	<.0001
60º LM - 90º LM	36.87	1.72	108	21.389	<.0001
0º EB - 0º LM	35.36	4.38	108	8.079	<.0001
60º EB - 60º LM	35.36	4.38	108	8.079	<.0001
90º EB - 90º LM	35.36	4.38	108	8.079	<.0001
0º LM - 60º EB	4.98	4.7	108	1.059	0.8964
60º LM - 90º EB	1.51	4.7	108	0.32	0.9995

Males (*N* = 49)

Contrast	**Estimate**	**SE**	**df**	***t* ratio**	***P-value***
0º EB - 90º LM	126.4	4.97	139	25.422	<.0001
0º EB - 60º LM	104	4.97	139	20.927	<.0001
0º EB - 90º EB	82.5	3.3	139	24.998	<.0001
60º EB - 90º LM	81.3	4.97	139	16.357	<.0001
0º EB - 0º LM	69.5	4.97	139	13.992	<.0001
60º EB - 60º LM	59	4.97	139	11.861	<.0001
0º LM - 90º LM	56.8	2.41	139	23.61	<.0001
0º EB - 60º EB	45.1	3.3	139	13.648	<.0001
90º EB - 90º LM	43.8	4.97	139	8.818	<.0001
60º EB - 90º EB	37.5	3.3	139	11.35	<.0001
0º LM - 60º LM	34.5	2.41	139	14.324	<.0001
60º EB - 0º LM	24.5	4.97	139	4.927	<.0001
60º LM - 90º LM	22.3	2.41	139	9.286	<.0001
90º EB - 60º LM	21.5	4.97	139	4.323	0.0004
0º LM - 90º EB	13	4.97	139	-2.612	0.1013

Figures

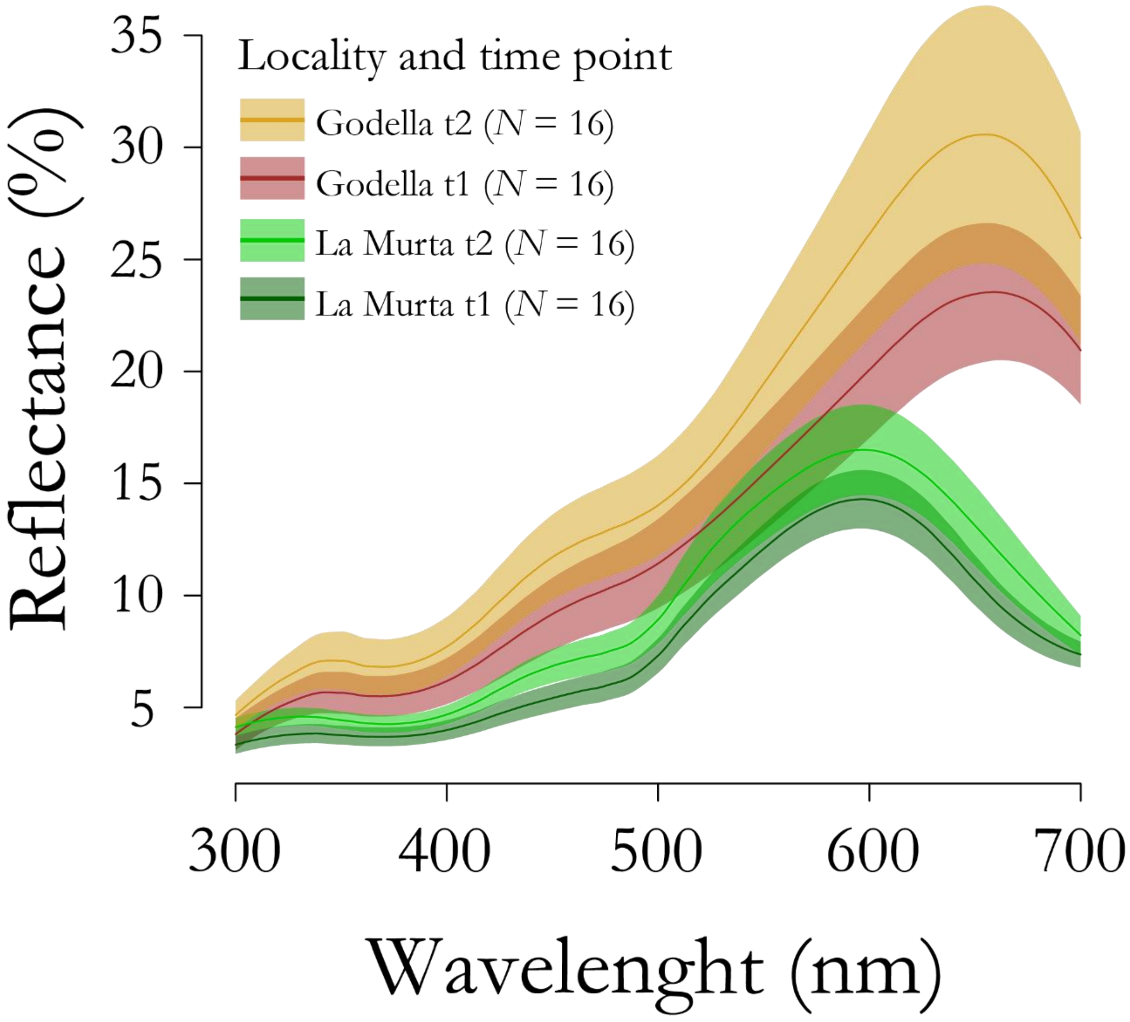

Figure S1. Repeated reflectance spectra of 16 males from LM showing green dorsal coloration and 16 males from Godella (6.3 km distant from EB) showing brown dorsal coloration. These lizards were housed in individual terraria for a period of six weeks, during which they were exposed to potentially stressful conditions (i.e. confronting one opponent per day in an agonistic interaction). For each lizard we measured dorsal reflectance before (t1) and after (t2) the experiment using standards methods detailed elsewhere (Font, Pérez i de Lanuza & Sampedro, 2009; Badiane *et al.*, 2017). Spectra were smoothed (span = 0.2) and normalized by subtracting the minimum value at all wavelengths. We observe a slight increase in luminance with time, but CI_{95} for the t1 and t2 spectra are largely overlapping. Chromatic aspects of coloration remain unchanged (i.e. wavelength of peak reflectance and pointiness of the spectral curve).

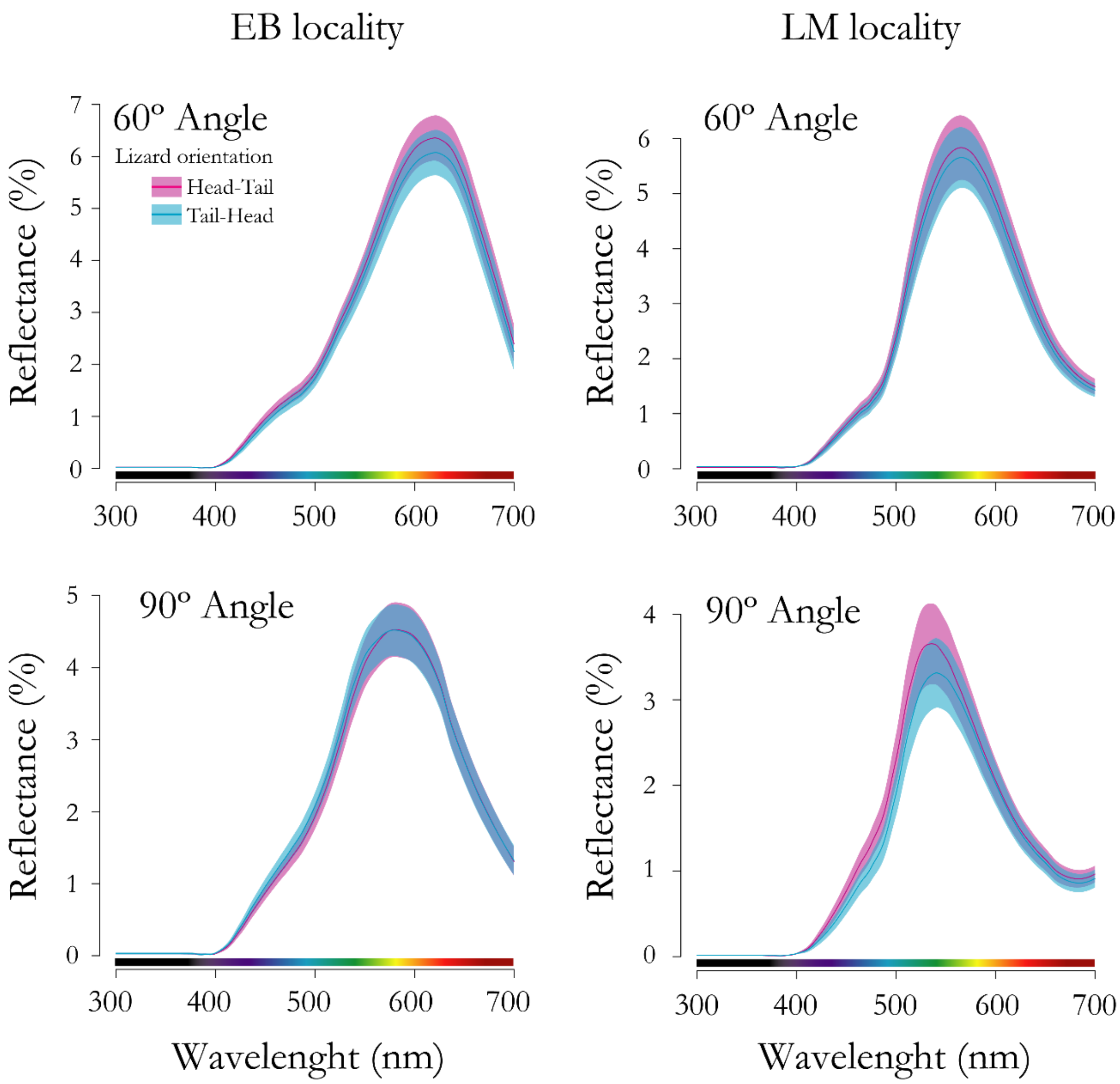

Fig. S2. Differences in reflectance between paired measurements of the same lizards taken with different lizard orientations (head-to-tail and tail-to-head), presented by locality and viewing angle. Reflectance spectra have been smoothed (span = 0.2) and normalized by subtracting the minimum value at all wavelengths from each individual spectra. CI_{95} (shaded areas) show a great degree of overlap between both sets of measurements, suggesting a minor impact for lizard orientation on measured reflectance while proving the high reliability of our methodology to measure reflectance at 60º and 90º. Upon observing only minimal differences, we used spectra averaged at the within-individual level for subsequent analysis.

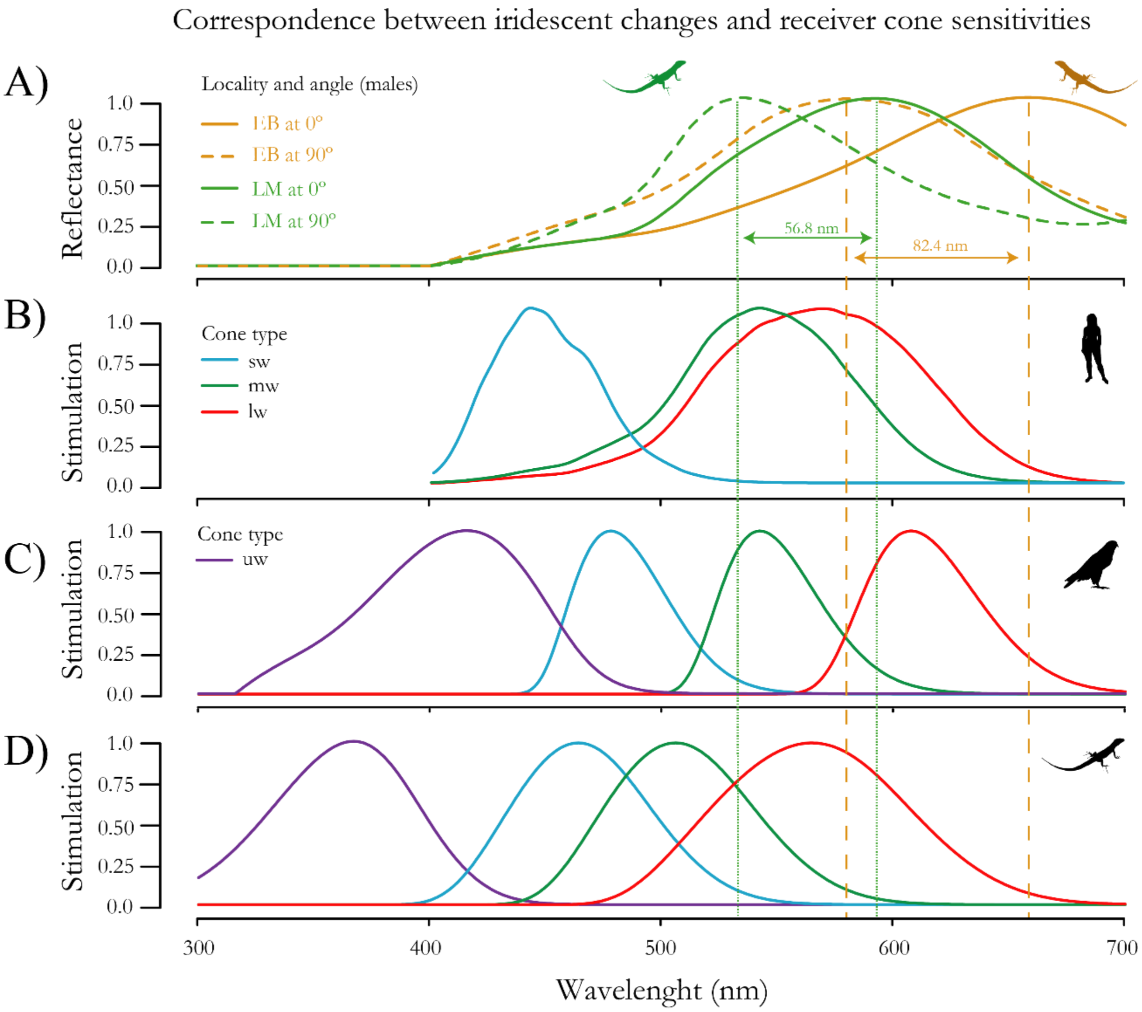

Figure S4. Correspondence between the iridescent spectral shifts in males from both localities (A) and the cone sensitivities used for modelling (B) human, (C) raptor, and (D) *Podarcis* vision. Vertical lines bound the waveband affected by peak shifts in LM (dotted green) and EB (dashed brown). Although iridescent spectral shifts are larger in EB than in LM, decreased overlap with receiver cone sensitivities results in lower sensory stimulation (i.e. shorter between-angle chromatic distances) in the former than in the latter (**Fig. 1**). Note however that the relationship between differential sensory stimulation and perceptual distance is often intricate and nonlinear.

Video V1. Two representative frames from Video V1, available as Supplementary material.

Cover Image suggestion