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RESEARCH ARTICLE

The importance of ultraviolet and near-infrared sensitivity for visual discrimination in two species of lacertid lizards

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ABSTRACT

Male and female Lacertid lizards often display conspicuous coloration that is involved in intraspecific communication. However, visual systems of Lacertidae have rarely been studied and the spectral sensitivity of their retinal photoreceptors remains unknown. Here, we characterise the spectral sensitivity of two Lacertid species from contrasting habitats: the wall lizard *Podarcis muralis* and the common lizard *Zootoca vivipara*. Both species possess a pure-cone retina with one spectral class of double cones and four spectral classes of single cones. The two species differ in the spectral sensitivity of the LWS cones, the relative abundance of UVS single cones (potentially more abundant in *Z. vivipara*) and the coloration of oil droplets. Wall lizards have pure vitamin A1-based photopigments, whereas common lizards possess mixed vitamin A1 and A2 photopigments, extending spectral sensitivity into the near infrared, which is a rare feature in terrestrial vertebrates. We found that spectral sensitivity in the UV and near infrared improves discrimination of small variations in throat coloration among *Z. vivipara*. Thus, retinal specialisations optimise chromatic resolution in common lizards, indicating that the visual system and visual signals might co-evolve.

KEY WORDS: Colour vision, Chromatic resolution, UV sensitivity, Vitamin A1/A2-based pigments, Cone abundance, *Zootoca vivipara*, *Podarcis muralis*

INTRODUCTION

Vision is a key sense involved in tasks such as mating, foraging and predator avoidance, and visual capabilities are expected to be optimised to the ecological niche of each species (Bradbury and Vehrencamp, 2011; Land and Nilson, 2012). Thus, it is of considerable interest to comprehend how animals perceive their environment and distinguish different visual targets. In vertebrates, photopic and colour vision are served by cone photoreceptor cells (see Bradbury and Vehrencamp, 2011). Photosensitivity is conferred by visual pigment molecules embedded in the membranes of the outer segments of retinal photoreceptor cells, and composed of a transmembrane opsin protein associated with a chromophore (for details, see Yokoyama, 2000). Photopigments are usually specified by the wavelength of peak absorbance, λ_{max}, and include long-wavelength sensitive (LWS class), middle-wavelength sensitive (MWS class), short-wavelength sensitive (SWS class) and very-short-wavelength sensitive (VS/UVS class) (Kelber et al., 2003). Colour vision requires the presence of at least two visual pigments differing in their spectral sensitivity as well as the neural and perceptual mechanisms capable of analysing and interpreting signals from the photoreceptors (Bowmaker, 2008). Characterisation of the spectral properties of the retina in various species is therefore a prerequisite for understanding the evolution of visual capabilities.

Spectral absorption of the visual pigments is determined by both the amino acid sequence of the opsin protein and the chromophore used, either the aldehyde of vitamin A1 or vitamin A2 (Bowmaker, 2008). Vitamin A1 is commonly encountered in the eyes of terrestrial vertebrates and marine species, whereas vitamin A2 is usually associated with freshwater species or the aquatic phase of terrestrial amphibians (reviewed by Bridges, 1972). For the same opsin protein, A2-based pigments (porphyropsins) show an absorption peak shifted toward longer wavelengths than the A1-based pigment (rhodopsins) (Hárosi, 1994; Whitmore and Bowmaker, 1989). It has been shown that some amphibian and fish species present individual plasticity in the relative proportion of A1- and A2-based visual pigments with age, hormonal state, light, temperature, season or life stage (Beatty, 1966; Beatty, 1975; Beatty, 1984; Crescitelli, 1972; Knowles and Darntnall, 1977). Some studies have found a chromophore mixture in lizards such as chameleons and *Podarcis sicula* (Bowmaker et al., 2005; Provencio et al., 1992) and, more surprisingly, *Anolis carolinensis* possesses a pure-cone retina containing only A2 pigments (Provencio et al., 1992; Loew et al., 2002). The adaptive significance of vitamin A1- versus A2-based visual pigment in the vertebrate retina is poorly understood.

A common feature of the retina of most diurnal reptiles and birds is the presence of pigmented oil droplets located in the distal region of the inner segment of cones, except for the accessory member of the double cones (reviewed by Bowmaker, 2008). Their lipid content and high concentration of carotenoid pigments act as a long-pass filter for the photons entering the outer segment, which shifts the sensitivity peaks of the photoreceptors to longer wavelengths. Oil droplets are believed to improve hue discrimination by restricting the range of wavelengths that enters the outer segment and reducing the overlap of spectrally adjacent cones (Stavenga and Wilts, 2014; Vorobyev, 2003). Previous studies in birds and lizards have demonstrated that each photoreceptor type can be associated with specific oil droplet types, based on its apparent colour to humans (e.g. Fleishman et al., 2011; Loew et al., 2002) [for examples in birds, see Hart and Vorobyev (Hart and Vorobyev, 2005)]. This specificity is particularly interesting because it allows indirect evaluation of the abundance of the different cone types and, therefore, part of the noise surrounding the response of a given photoreceptor type (Bradbury and Vehrencamp, 2011).

The majority of diurnal lizards are known to possess no rods and three or four spectral classes of photoreceptors (tri- or tetrachromats) including one photoreceptor sensitive to light in the UV range (300–400 nm) (reviewed by Pérez i de Lanuza and Font, 2014)

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List of abbreviations

C1	colourless type 1 oil droplet
C2	colourless type 2 oil droplet
DP	dispersed pigment
G	green oil droplet
JND	just-noticeable difference
LWS	long-wavelength sensitive
MSP	microspectrophotometry
MWS	medium-wavelength sensitive
O	orange oil droplet
SWS	short-wavelength sensitive
UV	ultraviolet
VS/UVS	very-short-wavelength sensitive
Y	yellow oil droplet

(supplementary material Table S1). There are also three to five spectral classes of oil droplets. One to three types of green and/or yellow (to the human eye) coloured oil droplets are paired with MWS and LWS pigments, and one or two types of colourless oil droplets are always associated with cells containing UVS and SWS pigments (Bowmaker et al., 2005; Loew et al., 2002; Pérez i de Lanuza and Font, 2014). Over the past decades, spectral absorbance of pigments has been investigated in several lizard species, but these species belong to a limited number of families and, to date, spectral sensitivity of several entire lizard infraorders remains essentially unknown (see supplementary material Table S1). Here, we focused on the Lacertidae family of the Lacertibaenia group, which includes most of the diurnal common European lizard species. Several lacertid species display coloured ornaments that differ between sexes, including in the UV range (e.g. Font et al., 2009; Martin et al., 2013). Even though olfaction plays a major role for foraging, navigation and communication in this family of lizards (see Mason and Parker, 2010), visual signals are also involved in intraspecific communication. Recent work in lacertids provided evidence for visual sensitivity to UV from retinal structure and molecular data (Pérez i de Lanuza and Font, 2014). In addition, behavioural tests indicate that lacertids can use UV signals of conspecifics to settle male contest and female mate choice (Bajer et al., 2010; Bajer et al., 2011).

The common lizard *Zootoca vivipara* Jacquin 1789 and the wall lizard *Podarcis muralis* Laurenti 1768 are interesting candidates for the study of visual systems of lacertids because the two species inhabit contrasting habitats, display bright, non-nuptial colour patches that reflect UV and use visual signals for intraspecific communication (Martin, 2013; Vacher and Geniez, 2010). The common lizard is commonly found in moist and grassy open habitats dominated by a green background. Males bear a whitish throat and a belly coloration ranging from yellow to dark red interspersed with black spots, and females are duller (Bauwens, 1987; Vercken et al., 2007). The ventral ornament also reflects in the UV range, especially on the throat of males which is exposed to conspecifics sight during agonistic interactions (Martin et al., 2013). The wall lizard inhabits stone walls and natural rock outcrops in open habitats dominated by a grey, highly reflective background. Adults of both sexes exhibit three ventral colour morphs (white, yellow and orange) (Galeotti et al., 2010; Sacchi et al., 2007) and males also have bright, UV–blue marginal ventral scales called blue spots that they exhibit by presenting their flank and by push-up displays (Pérez i de Lanuza, 2012; Martin, 2013).

In this study, we used microspectrophotometry (MSP) to determine the spectral absorbance of the visual pigments and oil droplets in *Z. vivipara* and *P. muralis*. From retinal photomicrographs, we also aimed to evaluate the relative abundance of the different oil droplet types, based on their colour for human eye. In both lacertid species, we found visual characteristics close to those of diurnal lizards studied so far. Nevertheless, *Z. vivipara* presented an A1/A2-based chromophore mixture and our data suggest that UV cones might be twice more abundant in *Z. vivipara* than in *P. muralis*. We thus used physiological data to model visual capabilities of the common lizard in order to investigate how the UV cone density and chromophore type affect chromatic resolution. This exercise helped us to gain further insight into the evolution of the visual system structure in lizard species by testing for optimisation of alternative visual systems against naturally occurring visual signals.

RESULTS

Spectral characteristics of lacertid lizards

We did not measure spectral properties of ocular fluid but our MSP analyses of the cornea revealed no significant absorption in the range 350–750 nm as in a recent analysis of eight lacertid lizard species (Pérez i de Lanuza and Font, 2014). The two study species possessed a pure-cone retina, which contained single cones with an oil droplet in their inner segment and double cones consisting of a principal member with an oil droplet and an accessory member with a dispersed pigment in its inner segment. In each species, four distinct single-cone classes were identified and were characterised as UVS, SWS, MWS and LWS. The details of pigment λ_{max} values of both species are provided in Table 1 (see supplementary material Figs S1 and S2 for representative examples). Absorption profiles of visual pigments from *P. muralis* were best fitted by a vitamin A1 template. In *Z. vivipara*, pigment absorptions were best fitted by a rhodopsin (vitamin A1) or a porphyropsin (vitamin A2) template, depending on the tested inner segment. Based on the absorption profile of LWS pigments, we estimated that vitamin A1- and A2-based pigments are a 10:90 proportion in *Z. vivipara*. However, this

Table 1. Characteristics of visual pigments found in cones of common and wall lizards

	Z. vivipara			*P. muralis*		
Pigment class	*N*	λ_{max}	Oil droplet	*N*	λ_{max}	Oil droplet
UVS (single)	4	358±8	C2	2	367±9	C2
SWS (single)	1	437	C1	3	456±23	C1
MWS (single)	20	487±14	O	3	497±19	G
LWS (single), form A1	2	544±4	G or O	11	562±17	Y or G
LWS (single), form A2	23	617±23		–	–	–
LWS (principal member of double)	6	614±17	G	1	584	Y
LWS (accessory member of double)	5	624±27	DP	1	558	DP

Number of counted cells, spectral sensitivity (mean λ_{max} ± s.d.) and associated oil droplet types for the different cone types. Because we could make a clear distinction between absorption profiles of LWS single cones fitted by a vitamin A1 or A2 template, the λ_{max} of each LWS pigment form is reported. Oil droplets belong to five classes: C1, C2, G, Y, O, plus a dispersed pigment (see List of abbreviations and Table 2).

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Table 2. Characteristics of oil droplets in retinal samples of common and wall lizards

	Z. vivipara			*P. muralis*		
Oil droplet class	N	λ_{mid}	% (Range)	N	λ_{mid}	% (Range)
Orange (O)	28	538±6	52 (15–71)	–	–	–
Green (G)	24	503±10	29 (13–63)	55	500±8	27 (22–42)
Yellow (Y)	–	–	–	5	470±4	64 (53–69)
Colourless, type 1 (C1)	9	406±9	19 (15–25)	4	429±22	9 (6–11)
Colourless, type 2 (C2)	4	+		2	+	
Dispersed pigment (DP)	2	485±11	–	7	460±11	–

Number and spectral features (λ_{mid} ± s.d., the wavelength at which the absorbance is 50%) of oil droplets measured by MSP and abundance based on retina images (as a percentage) were reported for each oil droplet type. Cut-off of the C2 droplets over the measurement range 340–750 nm is not measureable and thus a '+' indicates their presence in the cells of the retina.

estimate does not take into account potential variation of different retina regions; that is why we also used a 50:50 proportion in modelling in order to test the importance of this parameter in conspecific colour discrimination. It should be emphasised that template matching to MSP data is not the best way to assess chromophore type. However, it is safe to assume that if the λ_{max} of a measured pigment is greater than 580 nm, it most likely has an A2 component. For ecological studies, it is less important which chromophores are used because it is the spectral sensitivity of the cell that matters.

MSP allowed us to identify four spectral classes of oil droplet and one type of dispersed inner segment pigment in each species (see Table 2 for estimates of λ_{mid} of oil droplets and dispersed pigment). Both species possessed green oil droplets and two types of colourless oil droplet, and green oil droplets were on average less abundant than the other type of coloured oil droplets (Table 2; Fig. 1). *Z. vivipara* had orange oil droplets whereas *P. muralis* had yellow oil droplets (see supplementary material Fig. S3 for representative examples). In both species, one type of coloured oil droplets was exclusively associated with LWS pigments, but the second one was associated with both MWS and LWS pigment types, which impeded any estimate of the relative abundance of MWS and LWS cones. Data on the association between oil droplet classes and pigment classes are provided in Table 1. In the same way, colourless oil droplets were indistinguishable for a human viewer, and UVS and SWS cones cannot therefore be estimated from photographs. Counting of photoreceptors from retina photographs revealed 19% of colourless oil droplets in *Z. vivipara* but only 9% in *P. muralis*. Thus, the MSP data and oil droplet counts both suggest that UV cones are twice as abundant in *Z. vivipara* compared with *P. muralis*.

Quantitative modelling of visual performances of lacertids

The relative spectral sensitivity of each single cone class was calculated based on Hart and Vorobyev's templates (Hart and Vorobyev, 2005) for visual pigments and oil droplets, and is illustrated in Fig. 2 for both species. The spectral sensitivities of *Z. vivipara* and *P. muralis* were close in the spectral range between 300 and 480 nm, where the sensitivity of UVS and SWS cones had little overlap. By contrast, the range of sensitivity of MWS and LWS cones overlapped in both species. Because of the filtering effect of the oil droplet, the relative sensitivity of MWS cones was less than that of the other cones, especially in *Z. vivipara*. In addition, the retina of *Z. vivipara* displayed a wider range of sensitivity in long wavelengths than the retina of *P. muralis*, owing to the chromophore mixture observed in the LWS visual pigment. Given that the relative cone abundance cannot be precisely estimated, we used a rough estimate based on MSP data for the modelling exercise (UVS:SWS:MWS:LWS, 1:2:5:9). However, it should be noted that model outputs were almost identical when we assumed an equal abundances for MWS and LWS cones based on oil droplet counts (model 1:2:6:6, results not presented here).

Using the spectral data of ventral coloration of 84 adult male common lizards described in Martin et al. (Martin et al., 2013), we quantified the Cartesian distance in colour space for all possible pairs of males among spectra from the throat on one hand and from the belly on the other (3486 pair-wise comparisons for each body zone). The sample distribution of throat or belly colour distances for our MSP estimates (model with an A1/A2 chromophore mixture of 10/90 and cone ratios of 1:2:5:9, hereafter referred to as the empirical model) was characterised by a fat tail skewed to the right, a mode around 5 just-noticeable distance (JND) and <1% of the

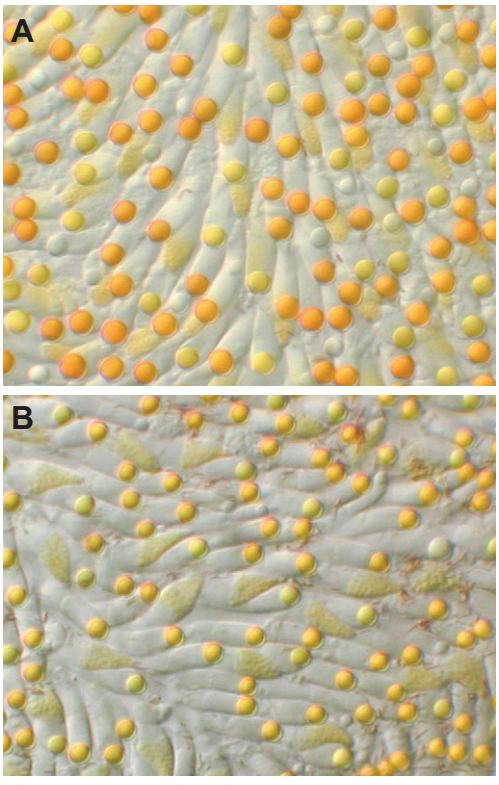

Fig. 1. Light microscopy of the retina of *Zootoca vivipara* and *Podarcis muralis*. Images of a small representative patch of the retina from (A) *Z. vivipara* and (B) *P. muralis*. Individual photoreceptors (elongated cells) and oil droplets are visible. Note the presence of two clearly distinguishable types of coloured oil droplets in both species and the abundance of colourless oil droplet in the retina of *Z. vivipara*.

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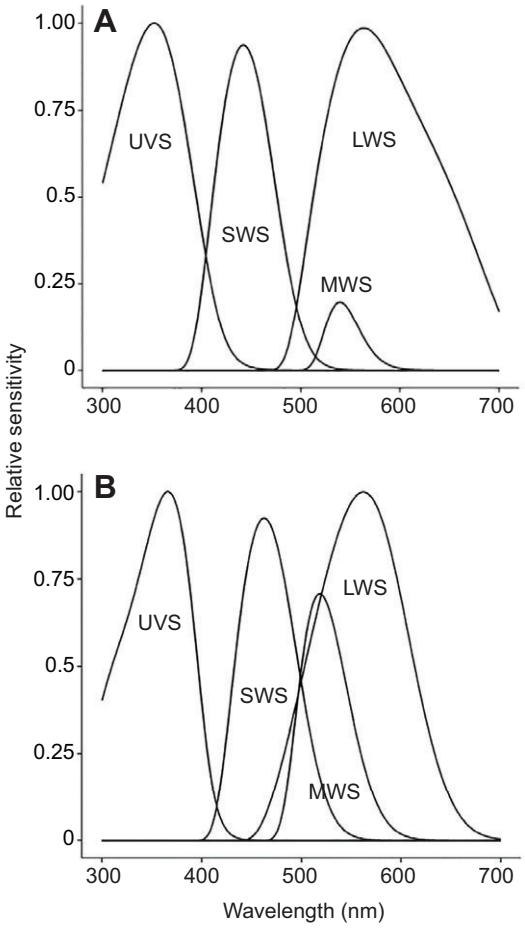

Fig. 2. Relative sensitivity of single cones in *Zootoca vivipara* and *Podarcis muralis*. (A) *Z. vivipara* and (B) *P. muralis*. Relative sensitivity was calculated as the product of the absorbance spectrum of visual pigments normalised to λ_{max} and the normalised transmission spectrum of their associated oil droplet.

distances less than 1 JND. Based on these observations, we then calculated the proportion of colour distances lower than 1 JND and those between 1 and 4 JND, and assumed that these distances are 'not distinguishable' and 'poorly distinguishable', respectively, in the subsequent analyses. Modelling results presented in Table 3 showed that very efficient discrimination of the belly colour patches, but slightly less discrimination of UV throat patches.

Comparisons between model outputs highlighted (Table 3), with respect to a visual system with cone types in equal ratios, the absence of sensitivity to UV light (trichromacy) strongly decreased the ability of the visual system of *Z. vivipara* to discriminate variation in throat and belly coloration. However, increasing the abundance of UV cones (model, 2:1:1:1) relative to other cone types decreased chromatic resolution slightly. Furthermore, with respect to a visual system with pure A1 pigments, a chromophore mixture in the retina of the common lizard enhanced chromatic resolution for the throat colour patch and, to a lesser extent, the belly colour patch. The outputs of the model with pure A2 pigments were similar to the output of the model with an A1/A2 mixture for throat data, and to the output of the model with pure A1 pigments for belly data.

DISCUSSION

Natural history data on the life style, foraging mode and anatomy of Lacertid lizards (Lacertidae) suggested to previous researchers that these species are more dependent on olfaction than on vision relative to other groups of lizards such as Iguanidae, Agamidae or Cordylidae (Mason and Parker, 2010; Vitt et al., 2009). Thus, we expected to discover atypical visual features in our two study species inhabiting contrasting habitats. However, common and wall lizards had visual properties of their retina similar to those seen in most diurnal lizards investigated so far (Barbour et al., 2002; Bowmaker et al., 2005; Ellingson et al., 1995; Fleishman et al., 2011; Loew, 1994; Loew et al., 2002; Macedonia et al., 2009). Interestingly, the visual system of *Z. vivipara* also presented some atypical features. First, we found that the LWS absorbance was best fitted by an A1/A2 chromophore mixture template, whereas most lizards studied so far use just A1. Second, an orange oil droplet was associated with the red-shifted LWS and the MWS visual pigment of *Z. vivipara* whereas this oil droplet is yellow or green in other diurnal lizard species. Third, the retina of *Z. vivipara* was potentially characterised by a high relative abundance in UVS cones, although this observation should be confirmed with more exhaustive MSP counts of cones and a higher sample size. The abundance and characteristics of oil droplets may indeed vary among individuals and between different regions of the retina (Fuller et al., 2003; Loew et al., 2002). We randomly sampled several regions of the retina, but our sample of lizards was too small to investigate inter-individual variation in this study.

Table 3. Chromatic discriminability between throat and belly spectra for the visual system of common lizards

	Throat contrast		Belly contrast	
Model parameter	<1 JND	1–4 JND	<1 JND	1–4 JND
Cone density				
0:1:1:1	10.70	40.68	1.86	19.77
1:1:1:1	1.92	27.63	0.20	8.38
2:1:1:1	3.16	37.14	0.52	13.91
1:2:5:9	**0.60**	**13.28**	**0.03**	**2.32**
A1/A2 ratio				
Pure A1	1.10	21.44	0.17	4.05
50/50	0.72	14.54	0.06	2.64
10/90	**0.60**	**13.28**	**0.03**	**2.32**
Pure A2	0.68	14.20	0.11	4.45

Cone density is expressed as UVS:SWS:MWS:LWS. JND, just-noticeable difference. Values are percentage of total throat or belly colour contrasts that are not discriminable (<1 JND) or poorly discriminable (1–4 JND) for models with a 10/90 proportion of A1/A2 LWS photopigments but different cone densities, and with the empirical cone density but different A1/A2 ratios. Lower percentages show higher discriminating ability of the viewer. Bold percentages correspond to the empirical model with the observed cone density and A1/A2 ratio.

Spectral sensitivity in lizards and the importance of near-infrared sensitivity

A review of the spectral data collected so far in lizard species (Fig. 3) highlights that interspecific variation in λ_{max} is small and of the same order of magnitude as intraspecific variation (supplementary material Table S1). The λ_{max} of UVS, SWS and MWS visual pigments in our two model species is also very similar to those recorded in the majority of other diurnal lizard species (Fig. 3). Thus, there appears to be little evidence of adaptive tuning of the spectral sensitivity of these visual pigments among lizard species in accordance with previous suggestions that these aspects of the vision physiology are strongly conserved (Archer, 1999; Fuller et al., 2003; Kröger et al., 1999). Nevertheless, available data also suggest significant variation in the spectral sensitivity of LWS

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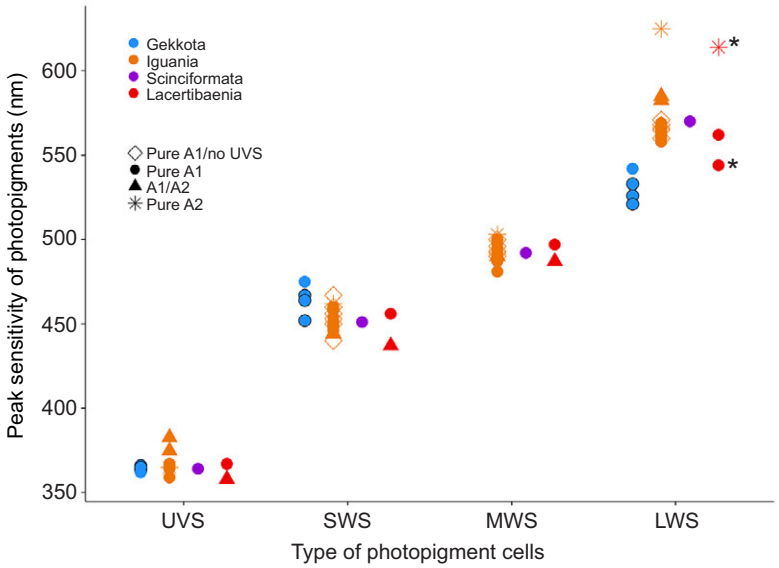

Fig. 3. Spectral sensitivity (λ_{max}) of visual pigments of lizard species for which MSP data are available to date. For each photopigment class, one data point corresponds to a species with point color referring to infraorder and point shape referring to visual system specificities [presence/absence of UVS cones and type(s) of chromophores]. All species are diurnal, except *Gecko gecko*, *Hemidactylus turcicus*, *Hemidactylus garnotii* and *Teratoscincus scincus* (blue dots with black outline). The λ_{max} of A1- and A2-based LWS pigments of *Z. vivipara* are reported separately (red dot and star with an asterisk, respectively). See supplementary material Table S1 for raw data.

single cones as well as variation in the abundance and type of oil droplets associated with single cones (supplementary material Table S1). Variation in the spectral sensitivity of LWS single cones was best attributed to the existence of vitamin A2 chromophores in *Z. vivipara* that extended spectral sensitivity into the near infrared (Archer, 1999; Hárosi, 1994; Whitmore and Bowmaker, 1989), whereas most diurnal lizards and terrestrial vertebrates use exclusively vitamin A1 chromophores in their visual pigments (Jacobs, 2010; Yokoyama, 2000). Vitamin A2 chromophore was previously recorded in *Anolis carolinensis* (Loew et al., 2002; Provencio et al., 1992) and a mixture of A1 and A2 chromophores was also shown by chromatography in *Podarcis sicula* (Provencio et al., 1992) and two chameleon species, *Chamaeleo dilepis* and *Furcifer pardalis* (Bowmaker et al., 2005). The presence of vitamin A2 in the eye of *Z. vivipara* remains to be confirmed by electrophysiology (Loew et al., 2002). San-Jose et al. (San-Jose et al., 2013) recently found that vitamin A2 was the dominant vitamin A compound in common lizards, where it is stored in the liver. They did not attribute this result to differential feeding but to a preferential synthesis and increased accumulation of vitamin A2 in *Zootoca vivipara*, which is usually absent in most species (San-Jose et al., 2013). Our results and findings in other lizard species thus suggest that the ability to synthesise vitamin-A2-based visual pigments sporadically appeared during the adaptive radiation of lizards.

Although it is clear that the nature of chromophores generates variation of visual sensitivity in lizards, it remains to be seen what advantage A2-based visual pigments provide. Compared with vitamin A1, vitamin A2 shifts absorbance of the visual pigment towards longer wavelengths (Hárosi, 1994), which may be optimal for intraspecific interactions under certain conditions. For example, Archer et al. (Archer et al., 1987) suggested that, in guppies, polymorphism in long-wavelength cones may be related to the ability to detect colour variations in the different yellow, orange and red spots used during sexual displays. In the same manner, a chromophore mixture as observed in *Z. vivipara* could ease the visual discrimination of small variations in the range of yellow-red colours, whereas pure A1- or A2-based pigment retina could narrow this range of sensitivity. Our model, however, predicted little effect of the type of chromophore on discrimination of yellow-red belly coloration. In fact, the yellow-red belly patch is strongly conspicuous (Bauwens, 1987; Martin, 2013; Vercken and Clobert, 2008) and fine-tuned chromatic resolution may not be necessary to detect inter-individual variations. By contrast, we found that a visual system based on a chromophore mixture outperformed a visual system with a pure A1 chromophore system and performed equally well as a pure A2 chromophore system in discriminating intraspecific variation of throat coloration. These results suggest that sensitivity in near infrared (i.e. presence of A2 chromophores) may be related to an appreciation of the differences in throat coloration of conspecifics. During behavioural displays, male common lizards expose their throat, but not their belly, to signal aggressiveness and dominance to other males and to attract females (Martin, 2013; Martin et al., 2013). Possession of a visual system sensitive to the near infrared may therefore allow better detection of slight differences between throat colour of conspecifics and, therefore, better assessment of the quality of a potential mate or rival (Martin, 2013).

Unexpectedly, MSP analyses also revealed atypical orange oil droplets associated with the red-shifted LWS and MWS visual pigments of *Z. vivipara* whereas they are yellow or green in other diurnal lizard species studied so far (supplementary material Table S1). Basically, oil droplets shift the sensitivity peaks of the photoreceptors towards longer wavelengths and narrow their spectral sensitivity functions (Stavenga and Wilts, 2014; Vorobyev, 2003). It is likely that the orange colour of oil droplets is an adaptation in response to the long-wavelength-shifted sensitivity of MWS and LWS photopigments due to A2 chromophores. However, among species with a chromophore mixture or pure A2 chromophores (supplementary material Table S1), *Z. vivipara* is the only one to show such a characteristic. Even though interspecific variation in transmission properties of the different types of oil droplets is not particularly noticeable (supplementary material Table S1), this discovery raises the interesting question of the adaptive significance of oil droplet colour (i.e. carotenoid pigments) which, to our knowledge, has not been addressed to date.

The importance of the abundance of UVS cones for lizard chromatic resolution

UV vision is common in lizards (Fleishman et al., 2011; Loew et al., 2002), including lacertids (Pérez i de Lanuza and Font, 2014). In many lizard species, social signalling encompasses colour patches with a UV component, and UV vision is thought to be tuned to

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detect small variability in the UV reflectance of conspecifics (Fleishman et al., 2011; Pérez i de Lanuza, 2012). We found UV-sensitive cones in both *Z. vivipara* and *P. muralis*, but our data also suggest that *Z. vivipara* might have twice as many UVS cones as *P. muralis*. Even though this difference could be an artefact due to our small sample size of cones in the MSP analysis, it raises the question whether the abundance of UVS cones is important for lizard chromatic resolution. Using a similar approach, Fleishman et al. (Fleishman et al., 2011) previously suggested that the superabundance of UV cones in the retina enhances discrimination of conspecifics during male–male competition in flat lizards *Platysaurus broadleyi*, because the throats of lizards from this species have small variations in UV reflectance that are easier to detect by a visual system where UVS cones are dominant. In the same vein, modelling of the spectral sensitivity of *Z. vivipara* showed that the presence of UV cones strongly improved visual performance for detecting small variations in the throat colour and, to a lesser extent, belly colour of conspecifics. This result is consistent with our expectations because both ornaments, but especially that of the throat, encompass a striking UV component and UV coloration plays an important role in sex recognition, mate choice and intra-sexual competition in this species (Martin, 2013).

Nevertheless, the model also predicted that a doubling in relative density of UVS cones decreased visual performance of common lizards. In the model, chromatic resolution is the consequence of sensitivity and relative abundance of pigments and their associated oil droplets as well as light environment and contrasts among colour patches (Kelber et al., 2003; Vorobyev and Osorio, 1998). Any increase in the relative abundance of one type of visual pigment is traded off against a decrease in the relative abundance of other visual pigments important for vision. Here, increasing the abundance of UVS cones relative to the cones sensitive to human visible light increased the detection of subtle inter-individual variation in UV coloration at the expense of the capability to detect variation in the yellow-red colour range. Given that the throat coloration of common lizards involves both structural (UV) and pigmentary (yellow-red) signals (Martin et al., 2013), the net effect on discrimination capacity was slightly negative when relative abundance of UVS cones got too high. Thus, an optimal relative abundance of UVS cones exists that maximises discrimination of colour patches involving dual visual signals.

Colour vision in diurnal lizards

Our study provides additional data on the visual systems of Lacertidae lizards, a widespread group of Squamate reptiles for which spectral sensitivity data had not been collected [except for UVS cones (see Pérez i de Lanuza and Font, 2014)]. Chemoreception is known to be an important sense in lacertids (Mason and Parker, 2010) and our results demonstrate that, at least in our two study models, lacertids also display a visual system similar to that of diurnal lizards, which is characterised by good chromatic resolution (Fleishman and Persons, 2001). These data confirm that there are few adaptations in diurnal lizards and, therefore, the ancestral visual system of this group appears to be relatively conserved (Archer, 1999; Fuller et al., 2003; Kröger et al., 1999) (supplementary material Table S1) giving rise to present day Squamate reptiles (Vidal and Hedges, 2009). Nevertheless, our study also suggest that some design components of visual sensitivity such as cone density, oil droplet colour and chromophore type may have evolved jointly with visual signals in order to maximise discrimination of differences in the colours of conspecifics that are important for social interactions.

MATERIALS AND METHODS

Study animals

In September 2011, at the end of the activity season, we captured four common lizards [*Zootoca* (*Lacerta*) *vivipara*, two males and two females] and four European wall lizards (*Podarcis muralis*, three males and one female) at the CEREEP-Ecotron IleDeFrance field station (France, 60 m above sea level, 48°17′N, 2°41′E). Adult common lizards were captured in enclosures located in a meadow where they can feed and behave as in natural populations. Adult European wall lizards were captured by noosing in a wild population living in the stone walls of the field station. After capture, each lizard was maintained in an individual terrarium littered with damp sand and wet mosses. After several days of accommodation, terraria were placed in the dark in a climate chamber. Temperature was then progressively cooled from 14 to 4°C during the first week and afterwards maintained constant at 4°C to mimic natural wintering conditions (Heulin et al., 2005). In February 2012, the temperature in the chamber was progressively increased over 48 h until it reached ambient temperature. Lizards were then removed from the chamber and maintained for 1 week in a terrarium provided with a light and heat source, a water dish, a shelter and live food. Afterwards, animals were shipped to the USA by air transport in a dark box and, upon arrival, were maintained in the same husbandry conditions as in France. All analyses were repeated in France in May 2013 using wild-caught animals (two adult individuals per species) to ensure that data were not biased by the use of animals emerging from hibernation. We found no obvious differences between the two samples or between the sexes, and thus pooled all data for our analysis. All protocols were approved by the French National Ethics Committee on Animal Experimentation (Comité National de Réflexion Ethique sur l'Expérimentation Animale, no. Ce5/2011/044).

Spectral absorbance of pigments and oil droplets

Microspectrophotometry was conducted by E.R.L. and protocols were the same as those described by Loew (Loew, 1994; Loew et al., 2002). We used four common lizards and four wall lizards (two individuals per year for each species, at least one female per species). After at least 2 h of dark adaptation, animals were anaesthetised with isoflurane, decapitated with sharp shears and the eyes enucleated under dim red light (safelight No. 2, 15 W bulb, Kodak, Rochester, NY, USA). Subsequent preparation and measurements were carried out under infrared illumination (>800 nm, Kodak safelight No. 11 or IR LEDs) using image converters. Eyes were hemisected, the cornea was isolated and the retinas carefully removed from the pigment epithelium under hypertonic buffer solution of Ca^{2+}/Mg^{2+}-free Ringer's solution at pH 7.2 supplemented with 6% sucrose. Pieces of retina were macerated, sandwiched between two coverslips edged with silicone grease, and placed on the stage of a computer-controlled single-beam MSP (Loew, 1994). Absorbance spectra were obtained for all clearly identified outer segments from 750 to 350 nm, and back again from 350 to 750 nm, with a wavelength accuracy of ~1 nm (Loew, 1994). Whenever possible, the inner segment of the same cell was also scanned to measure the absorbance of the oil droplet or dispersed inner segment pigment (the accessory members of the double cones). In some cases, it was not possible to scan the inner and outer segment for each cell and thus sample sizes for oil droplets and pigments differ. Post-measurement bleaching was used to confirm the presence of visual pigment. Corneal absorbance was measured from isolated pieces using essentially the same technique as for the retina.

Visual pigment λ_{max} was determined by template fitting using the method previously described by Loew et al. (Loew et al., 2002). Briefly, a Gaussian function was fit to the top 40 data points at 1 nm intervals and differentiated to establish the peak wavelength. The spectrum was normalised to this absorbance value and template fit to either A1 or A2 standard data using the method of MacNichol (MacNichol, 1986). Template fitting alone is not the best determinant of A1 or A2 status for noisy data such as that from the very small outer segments of diurnal reptiles. However, if the calculated λ_{max} was greater than 580 nm, it was assumed that A2 must be present. Calculated λ_{max} values are accurate to ±1.0 nm and are reported here to the nearest whole integer. Oil droplet and dispersed pigment absorbance spectra were plotted directly in units of optical density. For identification, the value of the wavelength at which the

absorbance is half way between the minimum and maximum values (λ_{mid}) was determined using the method of Lipetz (Lipetz, 1984).

Oil droplet abundance

In order to quantify the different types of oil droplets, we collected two small pieces of retina from each of three common lizards and three male wall lizards after anaesthesia. Samples were placed in drop of buffer and covered with a grease-edged coverslip and examined using an Olympus BHT microscope at ×40 magnification. Several images from different areas of each retina were captured and oil droplets were counted by eye from these images. In total, we counted around 200–800 oil droplets from each area. We did not attempt to score separately the different regions of the retina even though lizards may exhibit heterogeneous spatial distribution of their photoreceptors on the retina (New et al., 2012). However, our protocol ensured that we captured the average property of the eye. Associations between oil droplet classes and pigment classes were determined from data where the inner segment was attached to a droplet.

Body coloration measurements

We used the reflectance data of ventral coloration of adult male common lizards described in Martin et al. (Martin et al., 2013). Briefly, reflectance spectra were measured in the centre of the throat, chest and belly for 84 males in the early summer using a spectrophotometer (USB2000; Ocean Optics Inc., Dunedin, FL, USA) calibrated between 200 and 850 nm, a Xenon light source (PX-2) covering 220–750 nm and a 400 µm fibre optic probe (R400-7-UV/VIS, Ocean Optics Inc.). We restricted our analyses to 300–700 nm, which includes the broadest range of wavelengths known to be visible to lizards (Fleishman et al., 2011). The end probe in contact with the lizard's skin was bevelled at 45 deg and the circular reading spot was approximately 1 mm^2. Reflectance was measured relative to a dark and a white diffusive standard (WS-1, Ocean Optics Inc.). For each lizard, we measured two reflectance spectra for each body zone and calculated the average spectrum. Because spectral characteristics of chest and belly coloration were not significantly different (Martin et al., 2013), we used only throat and belly spectra in this study.

Quantitative model

We modelled visual signal perception by the common lizard using a version of the Vorobyev and Osorio model (Vorobyev and Osorio, 1998). This model assumes a receptor noise-limited colour opponent discrimination mechanism and can be parameterised with data on receptor spectral sensitivities, receptor abundance and noise levels in the photoreceptors [further details and applications are available for other species (see Osorio et al., 2004; Siddiqi et al., 2004; Vorobyev et al., 1998)]. This model has been successfully tested against behavioural discrimination tests in some birds, mammals and insects, but not in reptiles. In a nutshell, the model calculates relative quantum catch by each photoreceptor type according to data on light entering the eye and the spectral sensitivity of the receptor, including lens, ocular media and oil droplet absorption and visual pigment absorbance. For a tetrachromat, this calculation places objects seen under incident light into a calculated tetrahedral colour space (Goldsmith, 1990; Stoddard and Prum, 2008; Vorobyev et al., 1998). A threshold distance between two colours (i.e. the distance below which two stimuli are indistinguishable) can then be calculated following equation 5 in the Vorobyev and Osorio model (Vorobyev and Osorio, 1998), which assumes opponent mechanisms and noise in each receptor type. The distance in the tetrahedral colour space ΔS was calculated in units of multiples of just-noticeable difference (JND). A greater 'distance' in colour space between two colours indicates that these colours are easier to discriminate for a given visual system in a given environment. According to the opponent discrimination model, values of ΔS above 1.0 JND indicate that colours can be discriminated, whereas values below 1.0 indicate that colours are indistinguishable.

No data on photoreceptor noise is available for reptiles. Here, we assumed that receptor noise is independent of light intensity and used a Weber fraction of 0.05 as suggested for amphibians by Siddiqi et al. (Siddiqi et al., 2004). Relative sensitivity of single cones was calculated as the product of the normalised absorbance spectrum of visual pigments (outer segment) and of the relative transmission spectrum of oil droplets (inner segment) assuming a transparent lens and ocular media in the range 350–700 nm. For modelling purposes, we used Hart and Vorobyev's templates (Hart and Vorobyev, 2005) and estimates of λ_{max} from our MSP data to fit normalised absorbance spectra for each type of visual pigment. In addition, we used oil droplet templates from the same reference and estimates of λ_{mid} from our MSP data to calculate normalised transmission spectra of the oil droplets. These templates were designed for birds and there is no equivalent template for lizards. If both vitamin A1- and A2-based pigments were present in the mixture, the absorbance spectra of both types of pigments were calculated separately, multiplied by 0.5 and added before normalising and multiplying by the transmission spectra. We used a standard irradiance spectrum for daylight (D65 spectrum) (Wyszecki and Stiles, 1982) and all calculations of the model were run using Avicol version 6 (Gomez, 2006).

To evaluate the importance of the relative abundance of UVS cones, we ran the model on all possible pairs of throat spectra and of belly spectra from 84 male common lizards and calculated the value of ΔS for each of these throat or belly colour pairs. Analyses of throat and belly data were conducted separately because of their differences of spectral properties: the throat is rich in UV and poor in yellow-red pigmentation whereas the belly presents reverse colour properties. We were thus interested in the ability of the model to detect small colour variations for each colour patch. LWS pigments with an A1 or A2 chromophore were assumed to be in a 10:90 proportion. Four visual systems were tested: (1) no UVS cones (model 0:1:1:1); (2) UVS cones equal in abundance to other single cones (typical of most lizards, model 1:1:1:1); (3) UVS cones twice as abundant as the other single cones (based on UVS cones abundance observed in *Z. vivipara* relative to those in *P. muralis*, model 2:1:1:1); and (4) empirical estimates of the abundance of SWS, MWS and LWS single cones relative to UVS cones in *Z. vivipara* (model 1:2:5:9).

Furthermore, to explore the importance of the two chromophore types and their proportion, we ran the model on all possible pairs of throat or belly spectra from the 84 male common lizards. We tested four conditions by assuming the empirical cone density: (1) pure A1-based LWS pigments, (2) vitamin A1- and A2-based LWS pigments in 50:50 proportion, (3) vitamin A1- and A2-based long wavelength-sensitive pigments in 10:90 proportion (empirical estimate) and (4) pure A2-based LWS pigments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.M., J.-F.L.G., S.M. and E.R.L. designed and conceived the research; M.M. and E.R.L. performed the experiments; M.M. described and analysed the data; M.M. and J.-F.L.G. drafted the manuscript; M.M., J.-F.L.G., S.M. and E.R.L. revised the manuscript.

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Supplementary material

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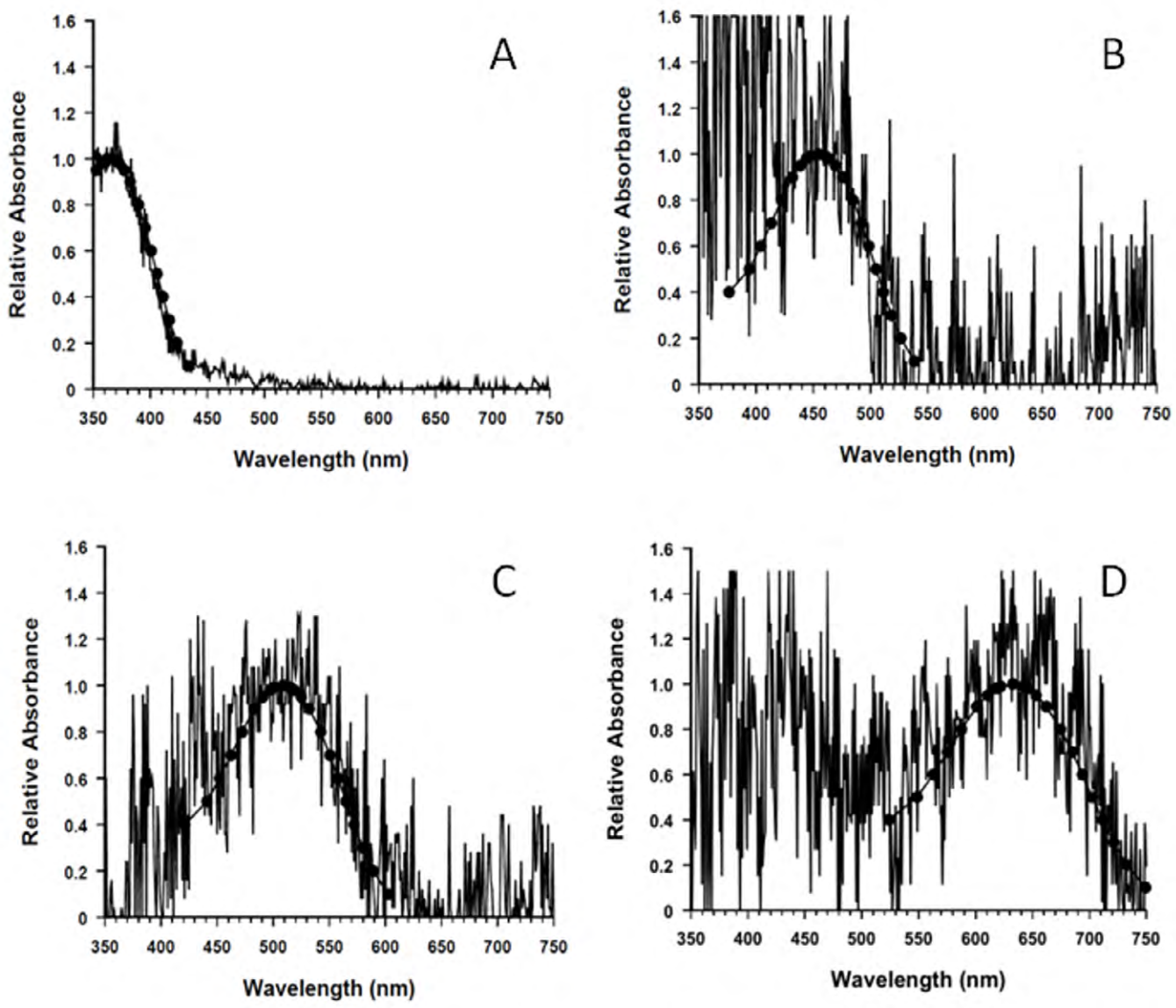

Fig. S1. MSP records in *Z. vivipara* of one representative (A) UVS, (B) SWS, (C) MWS and (D) LWS pigment. Curves correspond to the Gaussian function best fitted with the pigment absorbance profil.

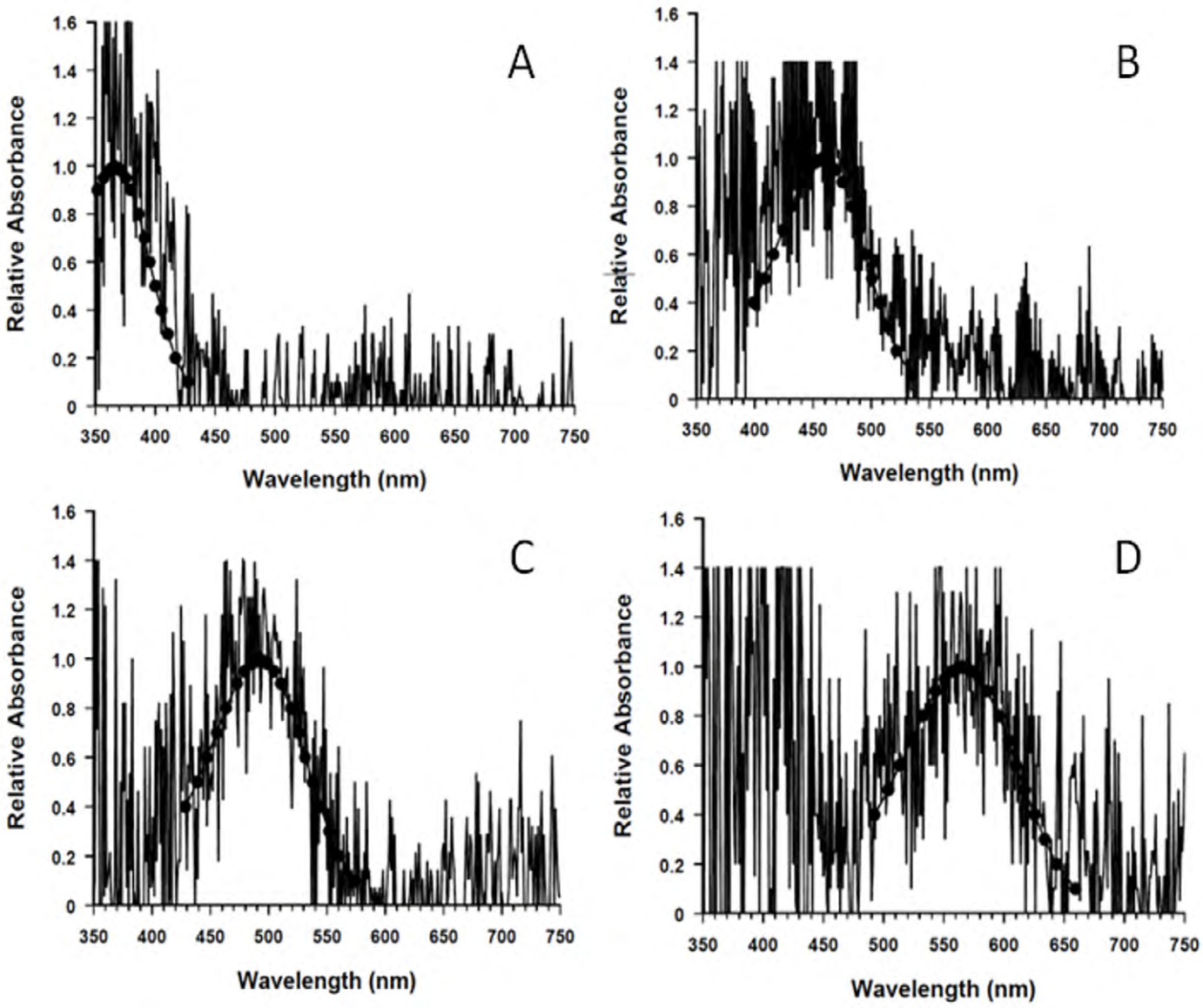

Fig. S2. Same as Fig. S1 for representative pigments in *P. muralis*.

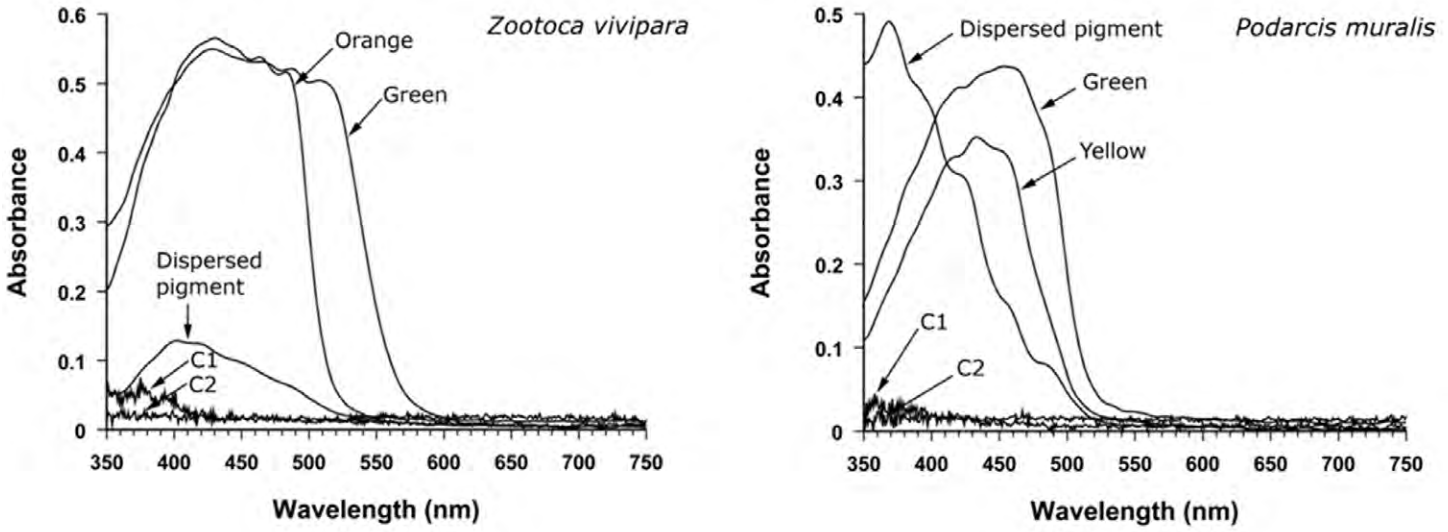

Fig. S3. Representative absorption spectra from MSP recordings of dispersed inner segment pigment and four classes of inner segment oil droplets in *Z. vivipara* and *P. muralis*. See Table 2 for quantitative data.

Table S1. Synthesis of MSP data on visual systems in squamate lizards. We reported the mean λ_{max} (nm) and λ_{mid} (nm) value ± s.d. (except when sample size is one) from the literature for photopigments and oil droplets, respectively.

		λ_{max} (nm) of photopigments				λ_{mid} (nm) of oil droplets			
Species name	Vitamine	UVS	SWS	MWS	LWS	G	Y	C	Reference
IGUANIA									
Anolis bahorucoensis	A1	365±6	450±6	500±6	569±4	500±6	450±7	397	Loew & al. (2002)
Anolis carolinensis	A2	365±5	462±5	503±8	625±3	507±4	463±5	365±2	Loew & al. (2002)
Anolis conspersus	A1	365±7	460±7	500±7	562±3	515±5	475±6	368±2	Loew & al. (2002)
Anolis cristatellus	A1	365±6	458±4	492±5	562±4	507±5	463±5	371	Loew & al. (2002)
Anolis equetris	A1	None	460±9	492±11	565±8	506±4	470±6	388	Loew & al. (2002)
Anolis evermanni	A1	364±5	460±5	490±3	565±3	515±7	500±6	380±4	Loew & al. (2002)
Anolis extremus	A1	365±7	451±7	487±9	566±5	488±5	442±4	393±3	Loew & al. (2002)
Anolis garmani	A1	None	467±10	496±9	565±8	492±4	466±5	371	Loew & al. (2002)
Anolis grahami	A1	367±8	460±6	495±7	565±6	505±10	451±6	382	Loew & al. (2002)
Anolis gundlachi	A1	365±7	450±9	490±7	564±5	510±4	450±6	370	Loew & al. (2002)
Anolis krugi	A1	365±5	448±6	490±5	562±4	500±5	480±5	370±3	Loew & al. (2002)
Anolis lineatopus	A1	366±5	449±2	498±4	560±2	486±8	451±4	367	Loew & al. (2002)
Anolis opalinus	A1	None	450±5	496±5	566±5	G1 521±3 G2 497±6	471±5	375±4	Loew & al. (2002)
Anolis pulchellus	A1	367±8	446±7	495±8	565±7	505±6	475	390	Loew & al. (2002)
Anolis sagrei	A1	365±3	460±6	495±5	567±4	510±5	475±3	376±2	Loew & al. (2002)
Anolis stratulus	A1	366±6	454±7	494±6	564±4	495±5	467±4	388	Loew & al. (2002)
Anolis valencienni	A1	None	456±8	500±8	560±9	522±2 505±4	479±4	368	Loew & al. (2002)
Crotaphytus dickersonae	A1	359±1	459±1	481±1	558±1	521±1	489±1	373±1	Macedonia et al. (2009)
Polychrus marmoratus	A1	None	453±5	490±3	568±4	G1 520±2 G2 485±5	462	368±2	Loew et al. (2002)
Ctenophorus ornatus	A1	None	440±1	493±9	571±4	Present	Present	C2 +	Barbour et al. (2002)
Chamaeleo dilepis	A1/A2	383±5	444±4	477/507	555/615	None	Y1 493; Y2 486	C1 390; C2 +	Bowmaker, Loew & Ott (2005)
Furcifer pardalis	A1/A2	375± 6	444±6	490±4	555/610	None	490	C1 390; C2 350	Bowmaker, Loew & Ott (2005)
GEKKOTA									
Gekko gecko	A1	364±3	467±2	None	521±1	N/A	N/A	N/A	Loew (1994)
Hemidactylus turcicus	A1	366	467	None	526	N/A	N/A	N/A	Loew et al. (1996)
Hemidactylus garnotii	A1	363	464	None	521	N/A	N/A	N/A	Loew et al. (1996)
Teratoscincus scincus	A1	365	452	None	533	N/A	N/A	N/A	Loew et al. (1996)
Gonatodes albogularis	A1	362±3	475±5	None	542±5	N/A	N/A	Present	Ellingson, Fleishman & Loew (1995)
SCINCIMORPHA									
Platysaurus broadleyi	A1	364±1	451±2	492±3	570±2	518±4	Y1 476±4 Y2 467±2	C1 380 C2 +	Fleishman et al. (2011)

All species are diurnal excepted *Gekko gekko*, *Hemidactylus turcicus*, *Hemidactylus garnotii* and *Teratoscincus scincus*. Photopigments UVS: ultraviolet-wavelength-sensitive, SWS: short-wavelength-sensitive, MWS: medium-wavelength-sensitive, LWS: long-wavelenth-sensitive. Droplets with green (G), yellow (Y) or colourless (C) oil. A same oil droplet class can present two spectral types referred to as, for example, G1 and G2. C2 oil droplets can have a λ_{mid} close or below the detection threshold of MSP, the presence of these oil droplets is then indicated by a '+' sign.