"Environmentally induced changes in carotenoid-based coloration of female lizards: a comment on Vercken et al" by Cote, J., Le Galliard, J.-F., Rossi, J.-M., and Fitze, P. S.

Supplementary material

Visual assessment of colour variation in female common lizards

Method 1 – Classification of Vercken et al. (2007)

We studied data that relied on the exact same methodology as Vercken et al (2007). Their colour reference to discriminate females in the field consisted of a cardboard painted with pale yellow, light green, intense yellow, pale orange, dark orange, pale red and dark red (see Photograph S1). Females were classified as showing pure colour, intermediate colour or mixed colour. In 1999, one of us (J-F L.G.) used this methodology to classify females from the same natural range as Vercken et al. (2007). Of the 156 females, 106 were classified as pure (61 pale yellow, 16 intense yellow and 28 orange), 49 as intermediate (10 pale yellow-yellow, 19 intense yellow-orange, 10 pale-orange) and 10 as mixed (2 pale yellow + yellow, 2 pale yellow + orange, 4 intense yellow + orange). These results show that most females had homogeneous coloration and that mixed yellow and orange females were rare (6 out of 156 individuals).

Photograph S1. Field assessment of female ventral coloration relied on a colour scale and visual inspection of coloration (Vercken et al., 2007). Photograph by J.-F. Le Galliard.

Method 2 – Visual inspection of digital pictures

In summer 2002, we took digital pictures of adult females captured in a natural population in the same geographic area as the one investigated by Vercken et al. (2007). These females did not

participate to the experiment described in this study but were analysed for their variation in ventral coloration. All adult females captured in the field were photographed on their belly following a strictly standardized procedure (Fitze & Richner, 2002). Control patches with red (RGB colour: 255,0,0) green (0,255,0), blue (0,0,255) and white (255,255,255) were fixed to each side of the lizard for calibration of the equipment during colour analysis: a quantitative analysis of the variation in light exposure as assessed from the measurements of the reference colours show highly consistent exposure conditions (Cote, unpub. data).

Some miniature digital photographs are provided below (see photo galleries 1 and 2). These pictures show variation in ventral coloration ranging from pale yellow to bright orange apart of some distinct brown pigmentation and the black belly spots. The yellow-orange ventral coloration typically expands from the thorax to the anal plate and it also expands on the throat in some orange females. The typical spatial patterning of coloration includes two internal longitudinal rows of scales characterised by homogeneous coloration and two external longitudinal rows of scales with more brownish and/or a lack of pigmentation. The coloration is often more intense close to the thorax and a decrease in coloration usually corresponds to more "brownish" pigmentation around the cloacae or on the flanks. In many cases females also lack pigmentation on the flanks, which usually starts a bit above the four middle lines of scales.

One of us (J-F L.G.) assessed the coloration heterogeneity of each scale within each of the four longitudinal rows from the thorax to the anal region (ca. 30-32 scales per row). Each scale was classified as homogeneous (H), slightly heterogeneous (SH), or obviously heterogeneous (OH) with distinct "yellow-like" and "orange-like" colour patches. Black spots were not taken into account and moulting females were excluded from this analysis. The proportion of scales in

each category is summarized for each line of scales and each ventral region in Table S1. The results show that scale heterogeneity was rare since obvious heterogeneity was restricted to 3-4% of the scales.

Table S1. Proportion of homogeneous, slightly heterogeneous and heterogeneous scales for ventral coloration in female common lizards (n = 86).

	Scale type	External left row	Internal left row	Internal right row	External left row
Thorax	H	83 %	84 %	84 %	82 %
	SH	15 %	13 %	14 %	16 %
	OH	2 %	3 %	2 %	2 %
Belly	H	82 %	84 %	84 %	86 %
	SH	14 %	12 %	11 %	10 %
	OH	4 %	4 %	5 %	4 %
Pelvis	H	77 %	79 %	79 %	81 %
	SH	20 %	17 %	16 %	16 %
	OH	3 %	4 %	5 %	3 %

Method 3 – Quantitative assessment of coloration in digital pictures

We also analysed the hue of the digital pictures on a randomly chosen 1 by 1 cm area from the thorax, the belly and between the thorax and the belly using PaintShop Pro software for image analysis. Hue values were normally distributed on each body part, the hue values were highly consistent across body parts (n = 86 adults, 3 measurements per individual; hue: $F_{85, 172} = 27.11$; $P < 0.001$; $r^2 = 0.93$) and the mean hue value on the belly measured with this methodology was well

correlated with the hue measured with a spectrophotometer on the thorax ($F_{1, 84} = 115.45$; $P < 0.001$; $r^2 = 0.58$). Finally, we analysed the colour parameters for 12 single scales uniformly distributed on the body (4 scales per region, 1 scale par row of scales). Vercken et al. (2007) defined the intermediate females as females with "intense yellow" or "mixture of yellow and orange". Therefore, females of mixed coloration should display higher heterogeneity in coloration among scales. Contrary to this expectation, the variances of hue and saturation among scales were higher for pale yellow females than for intermediate and orange females (Hue: $F_{2, 83} = 14.30$; $P < 0.001$; Saturation: $F_{2, 83} = 5.25$; $P = 0.007$; Brightness: $F_{2, 83} = 1.54$; $P = 0.22$). Based on these analyses, we can conclude that intermediate females did not display higher coloration heterogeneity among scales and that spectrophotometer measurements reflect well the redness of the ventral coloration of females.

References

Fitze, P. S. & Richner, H. 2002. Differential effects of parasite on ornamental structures based on melanins and carotenoids. Behavioral Ecology 13: 401-407.

Vercken, E., Massot, M., Sinervo, B. & Clobert, J. 2007. Colour variation and alternative reproductive strategies in females of the common lizard *Lacerta vivipara*. Journal of Evolutionary Biology 20: 221-232.

Photo gallery 1

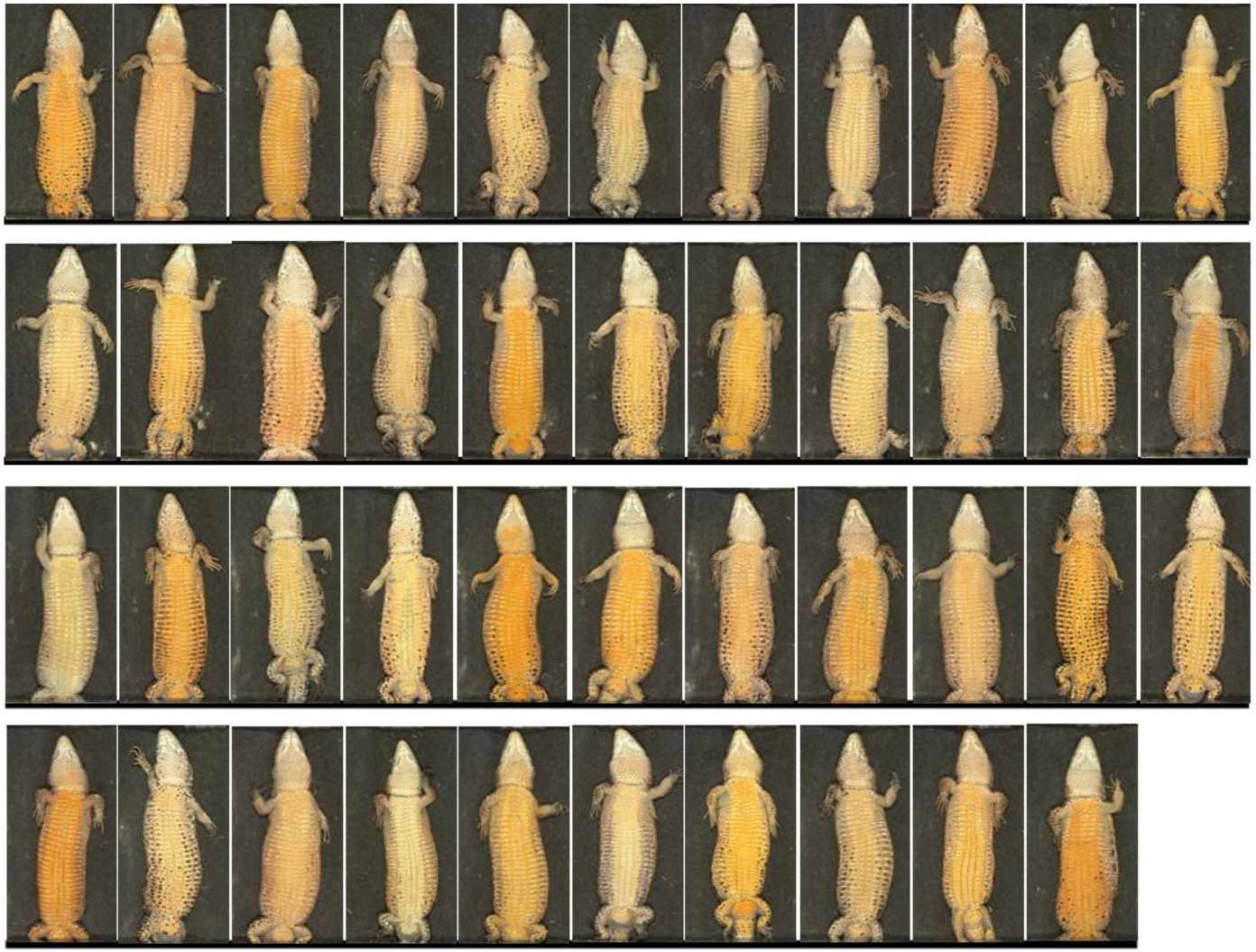

Photo gallery 2