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Head coloration reflects health state in the red-eared slider *Trachemys scripta elegans*

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Abstract Sexual signals can be evolutionarily stable if they are honest and condition-dependent or costly to the signaler. One possible cost is the existence of a trade-off between maintaining physiological health and elaboration of ornaments, such that only healthier individuals may afford to produce more elaborate sexual displays. We analyzed the relationship between head coloration and health state of *Trachemys scripta elegans* turtles. Results showed that turtles with a higher immune response and with a higher body condition had postorbital red patches with brighter coloration with higher values of long-wavelength reflectance (i.e., more reddish). Similarly, turtles with a higher immune response and with a lower heterophil/lymphocyte (H/L) ratio had chin yellow stripes with darker coloration with higher values of medium wavelengths (i.e., more yellowish). These relationships suggest that the health state of *T. scripta elegans* turtles is reflected by the colorful skin patches and stripes of the head. Characteristics of coloration did not differ between sexes, suggesting that this visual signal may be used by both sexes in intrasexual and intersexual communication. Because many other turtle species have similar colorful patches, it is likely that coloration may have a still unexplored significant role in sexual selection in many turtles.

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Introduction

Animal signals are expected to contain reliable information and to operate as honest indicators of health and quality, since they are costly to produce and maintain (Zahavi 1975; Grafen 1990; Johnstone 1995). Thus, only high-quality individuals can afford to exhibit honest signals, which can be used by conspecifics to accurately assess the status of potential mates or competitors (Andersson 1994).

In some cases, animals may incur important survival or physiological costs to generate striking signals that are sexually attractive. This can be particularly the case of some pigmentary colors that are also involved in several physiological functions. For example, carotenoid-based coloration systems of many vertebrates use pigments that have antioxidant and immunoregulatory properties, protecting cells and tissues from oxidative damage by trapping and detoxifying potentially harmful free radicals (reviewed in Lozano 1994; Olson and Owens 1998; Møller et al. 2000; Krinsky 2001; McGraw 2005). Consequently, healthy individuals that accumulate enough carotenoids could be able to allocate more pigments to ornamentation, hence signaling their quality to females, which may prefer to mate with the most colorful males (Hill 1990, 1991; McGraw and Ardia 2003). In fact, many studies have suggested that only healthier males may afford to produce more elaborate visual colorful displays (e.g., Blount et al. 2003; Faivre et al. 2003; Martín et al. 2008; López et al. 2009).

Carotenoids are by no means the only component of coloration. Color patches of vertebrates are considered multicomponent signals that often contain different classes of pigments and structural features, which have evolved through different

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ways (Endler 1992; Grether et al. 2004a). The basic mechanism of skin coloration in fishes, reptiles, and amphibians is the dermal chromatophore (Bagnara and Hadley 1973; Cooper and Greenberg 1992). This cell mainly consists of three contiguous layers: the xanthophore, containing carotenoid and pteridine pigments that absorb short-wave light; the iridophore, containing crystalline platelets that reflect color structurally; and the melanophore, which contains melanin that absorb light across the spectrum (Bagnara and Hadley 1973; Cooper and Greenberg 1992; Macedonia et al. 2000). Changes in the structure and concentration of these multiple components are associated with variation in physiological and behavioral traits (e.g., Fitze et al. 2009).

Since pigments must be ingested through the diet, coloration is, in many cases, closely related to foraging success and physiological efficiency of individuals (Grether et al. 1999, 2004a). In contrast with pigments that are deposited in feathers and other keratins that constitute inactive tissues, coloration expressed in active tissues such as the skin may respond rapidly to changes in physical condition, given that some pigments can be reallocated from the integument to serve immunological functions (Folstad and Karter 1992; Lozano 1994; Sak et al. 2003). Thus, color patches of the skin may signal current physiological and health state of individuals (Martín et al. 2008; Fitze et al. 2009), which can be useful in mate choice (Zuk et al. 1990; Lozano 1994; Olson and Owens 1998).

Many animal species exhibit multiple sexual ornaments which are used simultaneously to advertise their quality to rivals and to attract mates (Andersson 1994). Multiple sexual signals have been suggested to be redundant and reinforce one another conveying the same message to the receiver, or alternatively, to transmit different information of the overall quality of an individual (Møller and Pomiankowski 1993; Andersson et al. 2002; Pryke et al. 2002). A last hypothesis states that some male sexual ornaments have not arisen associated with female mate preferences or are Fisherian cues, not reliable indicators of physical condition (Møller and Pomiankowski 1993). Several studies suggest that only part of the multiple ornaments within a species is used in sexual selection (Zuk et al. 1990; Omland 1996). Hence, different signals might be directed at different kinds of receivers with different interests (i.e., either mates or competitors) and work as condition-dependent handicaps reflecting differing information and involving dissimilar associated costs. This possibility has been referred to as the "multiple receiver hypothesis" and constitutes an explanation for the evolution of several coexisting costly ornaments (Andersson et al. 2002).

Among reptiles, most studies testing the information content of multiple sexual signals have focused on lizards (e.g., Olsson 1994; Baird et al. 1997; Václav et al. 2007; Martín et al. 2008; Fitze et al. 2009; Martín and López 2009, 2010; Molnár et al. 2012), since many of them have evolved colorful sexually

dimorphic ornaments (reviewed in Cooper and Greenberg 1992). However, to our knowledge, no studies apart from Galeotti et al. (2011) have examined whether coloration is linked to genotipic or phenotypic quality in chelonians, despite some tortoises and many pond turtles present distinctive stripes and color patches on their heads, necks, and forelimbs (Ernst and Barbour 1989), which are susceptible of being condition-dependent traits working as honest signals.

Here, we analyzed the relationship between coloration and health state of a freshwater turtle, the red-eared slider, *Trachemys scripta elegans*. This is a semiaquatic medium-sized turtle original from the Mississippi basin, although it has been introduced worldwide. This turtle inhabits most freshwater systems such as lakes, marshes, ponds, and streams with abundant vegetation (Gibbons 1990). It is characterized by presenting a large postorbital red patch on each side of the head, varying from orange to an almost brick red color, and several narrow suborbital chin yellow stripes (Ernst and Barbour 1989). The red patch is larger in females than in males, and it can be completely darkened or disappear in old male specimens (Lovich et al. 1990a). In the rest of the body, the skin is green to olive brown with yellow stripes (Ernst and Barbour 1989). Physiological and behavioral data indicate that *T. scripta* has a very well-developed color vision (Ernst and Hamilton 1969; Granda et al. 1972), presenting one of the most complex eye cone system described for vertebrates (Ohtsuka 1985; Loew and Govardovskii 2001). During courtship, males vibrate their elongated fore claws on the female's head in a complex visual dance (Jackson and Davis 1972; Thomas 2002). In this position, both the male and the female show their head, neck, and forelimb patterns to each other. The presence of intense color patches in *T. scripta elegans* and the behavioral display during courtship suggest that this turtle might use multiple visual signals to communicate general and current health state to prospective mates and competitors.

In this paper, we measured the characteristics of coloration of multiple visual traits of adult *T. scripta elegans* turtles using spectrophotometry. We also estimated health state (i.e., immune response, white blood cell (WBC) counts, and body condition) of turtles because these variables may reflect health and be selected by turtles in potential mates (Ibáñez et al. 2012). Finally, we examined the relationships between characteristics of coloration and health state.

Materials and methods

Study animals

We obtained 37 red-eared sliders (carapace length: $\overline{X} \pm SE = 15.1 \pm 1.5$ cm) from the conservationist private organization Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat

(GREFA), where they had been maintained under seminatural conditions in a large outdoor pond. These sliders had been extracted from introduced populations in central Spain, with the purpose of preserving the original ecosystem balance. All turtles were individually housed at El Ventorrillo Field Station (Navacerrada, Madrid Province), in outdoor aquaria (60×40× 30 cm) filled with water and containing stones that allowed turtles to bask. Temperature and photoperiod were those of the surroundings (spring weather). Turtles were fed three times a week with mince, worms, and slugs. At the end of tests, all turtles had maintained or increased their body mass and were returned to the GREFA's pond. Procedures were conformed to recommended guidelines for use of live reptiles in laboratory research (ASIH 2004).

Health state

We assessed immune responses of turtles through relative WBC counts and T cell-mediated immune (CMI) responsiveness. High leukocyte counts have been interpreted to reflect an individual's current investment in immune defense (e.g., Møller 1998). In particular, elevated leukocyte count (leukocytosis) is characteristic of inflammatory processes in response to microbial and macroparasite infections (e.g., Dein 1986). Specifically, we concentrated on two leukocyte types, heterophils and lymphocytes. Heterophils are nonspecific phagocytosing cells that enter the tissues during the inflammatory response. Lymphocytes elicit pathogen-specific immune response. T lymphocytes (which comprise the majority of circulating lymphocytes) play a key role in CMI, while B lymphocytes that produce immunoglobulins are primarily responsible for antibody-mediated or humoral immunity (Kindt et al. 2007). An index comprising the relative abundance of both lymphocytes and heterophils is the heterophil/lymphocyte (H/L) ratio, which is widely used to estimate stress in poultry (e.g., Gross and Siegel 1983; Maxwell 1993) and also in wild birds (e.g., Birkhead et al. 1998; Totzke et al. 1999), lizards (Galeotti et al. 2010), snakes (Sperry et al. 2009), and turtles (Polo-Cavia et al. 2010a). The H/L ratio can be used to assess cellular immunocompetence because it increases in response to infectious diseases, inflammatory processes, or psychological disturbance (Maxwell and Robertson 1998; Ots et al. 1998; Sak et al. 2003; see review in Davis et al. 2008), although it has certain limitations (Smits 2007).

We prepared a smear on a microscope slide from blood taken from the caudal sinus at the base of the tail by using 1-mL syringe, 27 G, 1/2, 0.4×13 mm heparinized needle for each turtle. Blood smears were air-dried, fixed in absolute ethanol for 10 min, and then stained in Giemsa diluted 1:9 with phosphate buffer (pH 7.2) for 40 min before their examination for WBC. A single investigator counted the numbers of WBC, discriminating the different types of cells to estimate the H/L ratio. The proportion of different types of leukocytes was assessed on the basis of examination of 100 leukocytes at 1,000× magnification under oil immersion. Estimates of the total WBC were obtained by counting the number of leukocytes per 10,000 erythrocytes. Differential leukocyte counts were obtained by multiplying their proportions with WBC. The repeatabilities of leukocyte counts obtained with this method were found to be reasonably high and significant (Ots et al. 1998).

In addition to leukocyte counts, we also employed another commonly used technique to assess the immune response, the phytohaemagglutinin injection assay (PHA test). This test is considered as a reliable measure of T cell-dependent immunocompetence in vivo (McCorkle et al. 1980; Lochmiller et al. 1993), which has been used in many studies of animals including reptiles (Svensson et al. 2001; Belliure et al. 2004; Sacchi et al. 2007; Huyghe et al. 2010) and turtles (Polo-Cavia et al. 2010a; Zimmerman et al. 2010; Schwanz et al. 2011). Subcutaneous injection with PHA induces a complex series of physiological reactions, producing local inflammation (Chandra and Newberne 1977). Because it produces only a brief, local reaction, it is not thought to be of the physiological level, as PHA is a powerful mitogen, which activates WBC in peripheral blood, besides causing temporary inflammation at the point of injection (Roitt et al. 1996). Nevertheless, although PHA-induced swelling is related to heightened immune cell activity, it also may involve both innate and adaptive components of the immune system (Martin et al. 2006). Thus, this test does not appear to be an unambiguous index of CMI per se but rather a multifaceted index of cutaneous immune activity that is initiated by T cells. However, in this study, we used this test because we were interested in using swelling as a standardized index of immunocompetence, independently of the type of immune cells involved in the response. The CMI response is dynamic and may change seasonally in turtles (Zimmerman et al. 2010; Schwanz et al. 2011), but we were interested in the relationship between CMI and coloration during the reproductive season.

We measured CMI responsiveness after blood extraction to avoid possible effects of the immune test on relative WBC counts. CMI responsiveness was estimated on the basis of quantification of the swelling response to intradermally injected PHA (Smits et al. 1999). We injected each turtle's footpad of the left hind limb with 0.02 mg PHA solution (PHA-P, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.01 mL of phosphate-buffered saline and measured the swellings with a pressure sensitive spessimeter (Mitutoyo, Aurora, IL, USA) to the nearest 0.01 mm before and 24 h after the injection. The only appreciable effect of the PHA injection was a slight swelling of the skin due to the immune response. Previous tests with a similar terrapin species indicated that repeatability of these measurements was high (Polo-Cavia et al. 2010a).

We also measured and weighed all turtles. We used a metric tree caliper (Haglof, Madison, MS, USA) to measure body (shell) dimensions to the nearest millimeter and a digital electronic balance (Fisher Science Education, precision: ±0.1 g) for body mass. Maximum carapace length was measured as the greatest straight line distance from the anterior end to the posterior end of the shell. The residuals of an ordinary least-squares linear regression of log-transformed mass against log-transformed carapace length were used as a body condition index. It has been argued that such residuals provide the cleanest way to separate the effects of condition from the effects of body size (see reviews in Green 2000; Schulte-Hostedde et al. 2005).

Coloration measurements

We measured reflectance of head coloration from 300 to 700 nm using an Ocean Optics USB2000 spectroradiometer with a DT-1000-MINI Deuterium-Halogen light source (Ocean Optics, Inc., Dunedin, FL, USA). To exclude ambient light and standardize measuring distance, a cylindrical metallic tube was mounted on the bifurcated fiber optic probe (Montgomerie 2006). The probe was held at a 90° angle to the skin, and reflectance was measured, always by the same person. We measured coloration on two standardized spots of each turtle: the center of the widest area of the postorbital red ear patch (right and left) and the central area of the widest yellow (or cream-colored) suborbital chin stripe (right and left). Right and left measurements were highly repeatable within spots (intraclass correlation coefficients, $r > 0.83$, $P < 0.0001$ in all cases), so we calculated the mean values of both sides of the head for each turtle. Reflectance (R) was calculated relative to a white standard (WS-1-SS) with the OOIBase32 software (Ocean Optics, Inc.). Mean reflectance was summarized over 6-nm steps ('binned'; Grill and Rush 2000) before statistical analysis.

Statistical analysis

We mathematically summarized the spectra using principal component analysis (PCA) (Cuthill et al. 1999; Grill and Rush 2000). This method makes no assumptions about how reflectance variation is perceived or which aspects of the spectrum might be important (Cuthill et al. 1999). In PCA of spectral data, PC1 represents variation in intensity of coloration or brightness and subsequent PCs represent combinations of hue and chroma (Cuthill et al. 1999; Grill and Rush 2000). Also, the PCA identifies those sections of the spectrum (wavelength regions) that are contributing to the observed variation, independently of their "importance" in terms of contribution to the total amount of reflectance (Montgomerie 2006). Two PCA were performed separately

including all spectra for the postorbital red patches and those for the suborbital yellow stripes, respectively.

To determine the relationships between variables describing health state (H/L ratio, CMI response, or body condition) and coloration, we used backward stepwise general regression models (GRM) in STATISTICA Software (StatSoft, Inc., Tulsa, OK, USA) with these health state variables as dependent variables and PC scores describing coloration of red patches or yellow stripes as independent variables.

Results

Characteristics of coloration

Postorbital red patches

The PCA on reflectance data of all spectra of coloration of the postorbital red patches (Fig. 1a) produced three principal components (poPCs) that together accounted for 96.0 % of
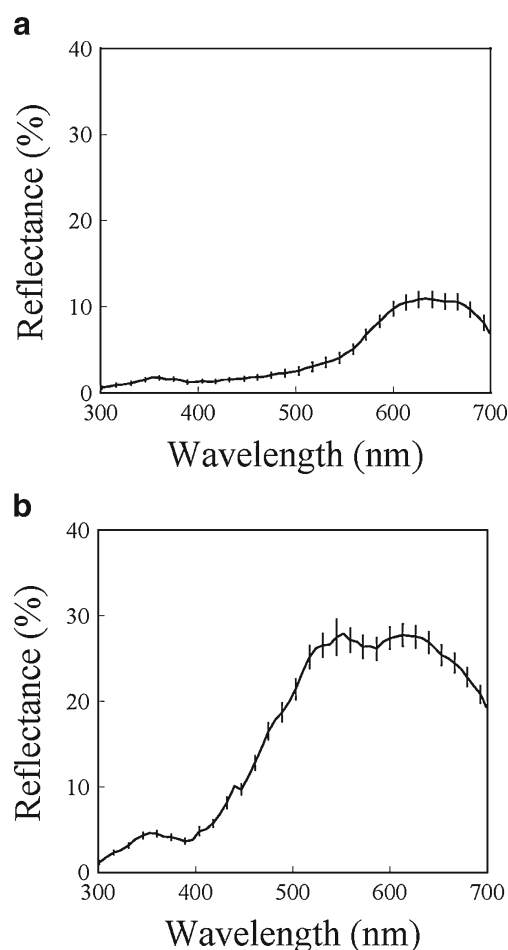
Fig. 1 Mean (±SE showed at 15-nm intervals) reflectance spectra of (a) postorbital red patches and (b) suborbital chin yellow stripe coloration of *T. scripta elegans* turtles

the variation in the original spectra. The first PC (poPC-1) accounted for 64.6 % of variation (eigenvalue=37.44). The coefficients relating poPC-1 to the original reflectance data were all negative and of similar magnitude (Fig. 2a), so poPC-1 represented achromatic brightness variation in the original spectra. The second PC (poPC-2) accounted for a further 17.3 % of the variation (eigenvalue=10.06) in the original spectra. The coefficients relating poPC-2 to the original reflectance values below 490 nm were all positive, while above 490 nm, they were negative (Fig. 2a). poPC-2, thus, represented variation in the relative amount of short- to long-wavelength reflectance. The third PC (poPC-3) accounted for 14.1 % of the variation (eigenvalue=8.16), and the pattern of coefficients suggested it represented

a

Fig. 2 Coefficients of the first three principal components from PCAs on reflectance spectra that characterize (**a**) postorbital red patches and (**b**) suborbital chin yellow stripe coloration of *T. scripta elegans* turtles

variation in the relative amounts of medium (430–580 nm) wavelengths in the positive side to both short (300–480 nm) and long (580–700 nm) wavelengths in the negative side (Fig. 2a).
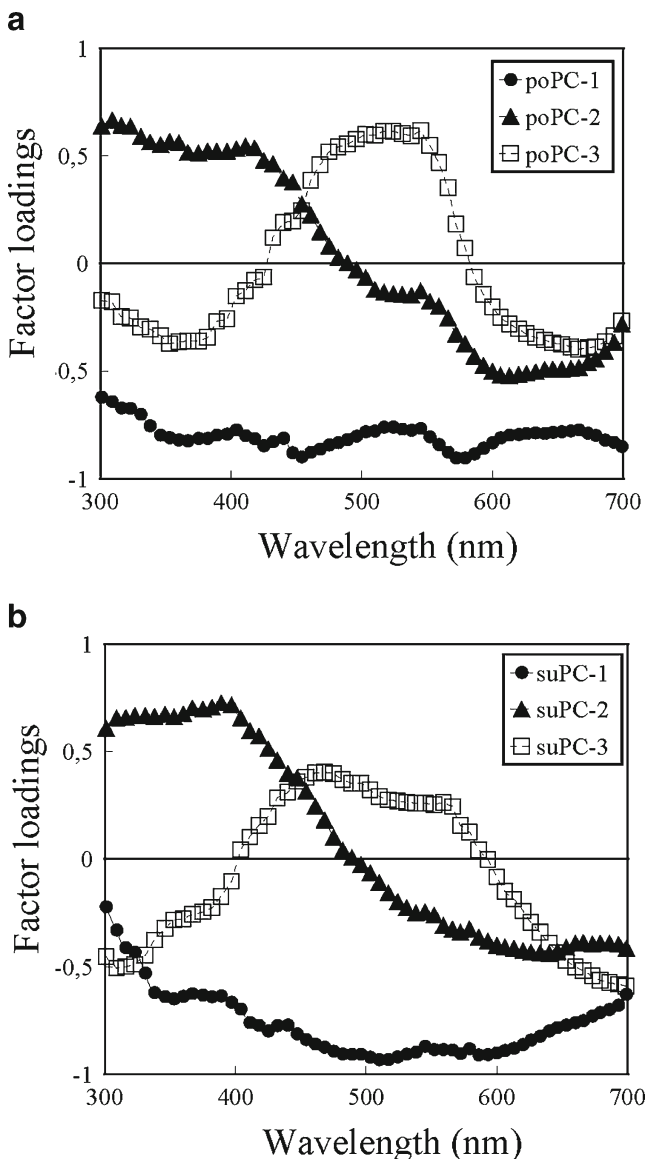
Suborbital chin yellow stripes

The PCA on reflectance data of all spectra of coloration of the suborbital chin yellow stripes (Fig. 1b) produced three principal components (chPCs) that together accounted for 94.6 % of the variation in the original spectra. The first PC (chPC-1) accounted for 60.4 % of variation (eigenvalue=35.06). The coefficients relating chPC-1 to the original reflectance data were all negative and of similar magnitude (Fig. 2b), so chPC-1 represented achromatic brightness variation in the original spectra. The second PC (chPC-2) accounted for a further 20.4 % of the variation (eigenvalue=11.82) in the original spectra. The coefficients relating PC-2 to the original reflectance values below 490 nm were all positive, while above 490 nm, they were negative (Fig. 2b). chPC-2, thus, represented variation in the relative amount of short- to long-wavelength reflectance. The third PC (chPC-3) accounted for 13.8 % of the variation (eigenvalue=7.99), and the pattern of coefficients suggested it represented variation in the relative amounts of medium (400–590 nm) wavelengths in the positive side to both short (300–400 nm) and long (590–700 nm) wavelengths in the negative side (Fig. 2b).
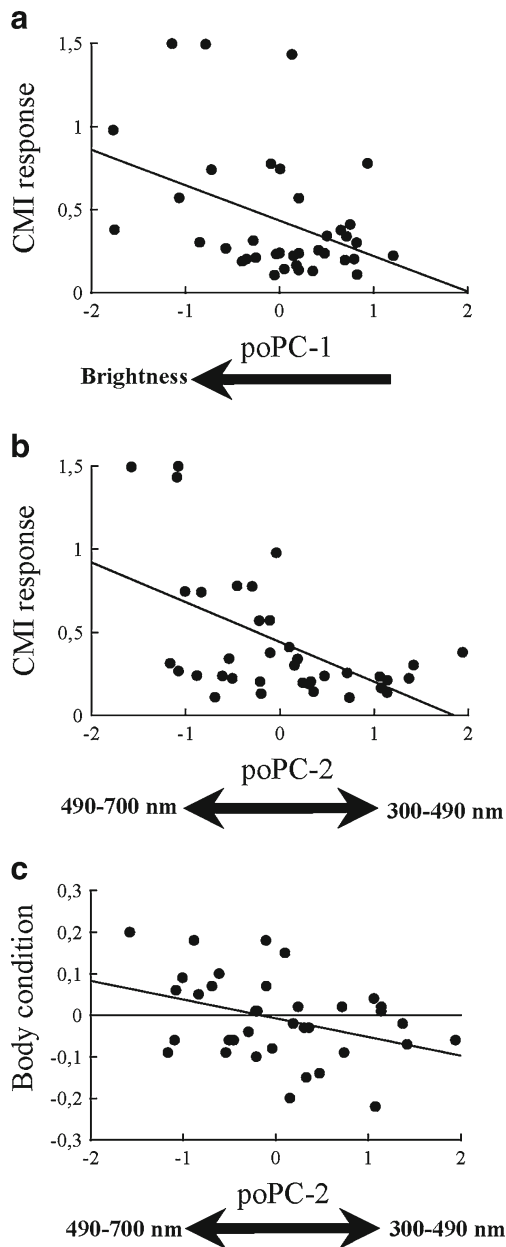
Sex differences

There were no significant differences between males and females in characteristics of coloration of postorbital red patches (ANOVAs, poPC-1: $F_{1,35}=0.60$, $P=0.44$; poPC-2: $F_{1,35}=0.20$, $P=0.66$; pohPC-3: $F_{1,35}=1.07$, $P=0.31$) or chin yellow stripes (ANOVAs, chPC-1: $F_{1,35}=0.01$, $P=0.97$; chPC-2: $F_{1,35}=0.11$, $P=0.74$; chPC-3: $F_{1,35}=2.92$, $P=0.096$). Also, including sex and its interactions to the GRMs models did not alter any of the results (data not shown). Therefore, we pooled males and females for further analyses.

Relationships between coloration and health state

With respect to coloration of the postorbital red patches, the immune response (CMI) was significantly and negatively correlated with poPC-1 and with poPC-2 (stepwise GRM, model: $R^2=0.47$, $F_{2,34}=15.31$, $P<0.0001$; poPC1: $\beta=-0.44$, $t=-3.51$, $P=0.0012$; poPC2: $\beta=-0.54$, $t=-4.37$, $P=0.0001$). Thus, turtles with a higher immune response had postorbital red patches with brighter coloration and with higher values of long-wavelength (>490 nm) reflectance (Fig. 3a, b). The H/L ratio was not significantly related to any poPC describing postorbital red patch coloration. Thus,

a

b

c

correlated with chPC1 (stepwise GRM, model: R^2=0.22, $F_{1,35}$=9.77, P=0.0035; chPC1: β=0.47, t=3.13, P= 0.0035). Thus, turtles with a higher immune response had lower brightness chin yellow stripes (Fig. 4a). Similarly, the H/L ratio was significantly and negatively correlated with chPC1 and with chPC3 (stepwise GRM, model: R^2=0.27, $F_{2,34}$=7.66, P=0.002; chPC1: β=−0.49, t=−3.47, P= 0.0014; chPC3: β=−0.30, t=−2.12, P=0.04). Thus, turtles with a lower H/L ratio had lower brightness chin yellow stripes and with higher values of medium wavelength (400–590 nm) reflectance (Fig. 4b, c). Body condition was not
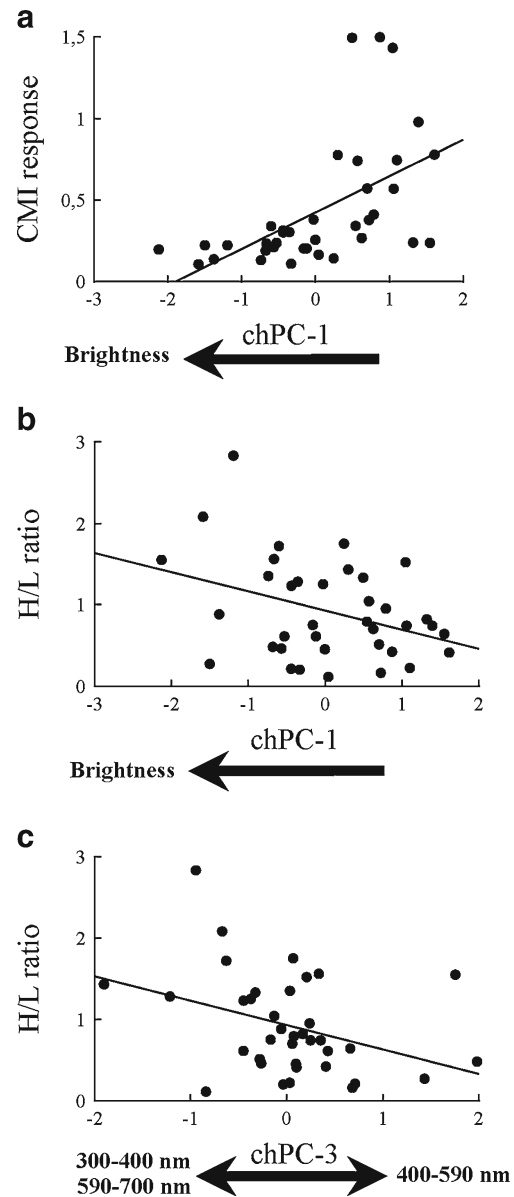
a

b

c

Fig. 3 Relationships between (**a**) and (**b**) the cell-mediated immune (CMI) response or **c** the body condition index with the poPC scores describing characteristics of postorbital red patch coloration of *T. scripta elegans* turtles. *Arrows* indicate the variables or wavelengths correlated with each PC

coloration characteristics of red ear patches seemed independent of the H/L ratio. Body condition was significantly and negatively correlated with poPC2 (stepwise GRM, model: R^2=0.15, $F_{1,35}$=5.97, P=0.02; poPC2: β=−0.38, t=−2.44, P=0.02). Thus, turtles with a higher body condition had postorbital red patches with coloration with higher values of long-wavelength (>490 nm) reflectance (Fig. 4c).

With respect to coloration of the chin yellow stripes, the immune response (CMI) was significantly and positively

Fig. 4 Relationships between a the cell-mediated immune (CMI) response or (**b**) and (**c**) the heterophil/lymphocyte (H/L) ratio with the chPC scores describing characteristics of suborbital chin yellow stripe coloration of *T. scripta elegans* turtles. *Arrows* indicate the variables or wavelengths correlated with each PC

significantly related to any chPC describing chin yellow stripe coloration.

Discussion

Our study suggests that the physiological health state of *T. scripta elegans* turtles is reflected by the colorful skin patches and stripes of the head. Turtles with brighter and more "reddish" postorbital patches have better health as indicated by a higher immune response and higher body condition. Similarly, turtles with darker and more "yellowish" chin stripes have better health as indicated by a higher immune response and lower H/L ratio.

These health-dependent variations in characteristics of coloration are similar to those observed in other animals that have colorful ornaments (e.g., Blount et al. 2003; Faivre et al. 2003; McGraw and Ardia 2003; Sak et al. 2003), including other reptiles, such as lizards (Václav et al. 2007; Galeotti et al. 2010; Molnár et al. 2012) or chelonians (Galeotti et al. 2011). For example, less parasitized male *Podarcis muralis* lizards had brighter and more yellowish ventral colorations, and males with a greater immune response had more saturated coloration (Martín et al. 2008). Males of two related "green" lizard species with a higher immune response have more greenish, darker, and more saturated dorsolateral colorations (Martín and López 2009, 2010). These results suggest the existence of a trade-off between physiological regulation of the immune system and the allocation of essential compounds (probably carotenoids) to sexual color ornaments. Reproductively mature turtles *Chrysemys picta* have reduced lymphocytes compared with juvenile turtles in the spring, presumably driven by a trade-off between maintenance and reproduction (Schwanz et al. 2011), which could include costs of producing colorful ornaments.

Experimental support to this trade-off has been provided in birds (Blount et al. 2003; Faivre et al. 2003; McGraw and Ardia 2003), fishes (Grether et al. 2004b), and lizards (López et al. 2009). This is explained because carotenoids are important in the upregulation of various aspects of the immune functioning and cellular protection against oxidation by free radicals (Lozano 1994; Krinsky 2001; McGraw 2005). As animals may suffer a trade-off between allocation of pigments to physiological functions and ornamentation (Faivre et al. 2003; McGraw and Ardia 2003), the expression of coloration may serve as an honest signal of individual quality (Møller et al. 2000; Blount et al. 2003), which may have an important role in both intra- and intersexual selection processes.

Interestingly, information provided by chin stripes and postorbital patches seem different. Both signals are related to the immune response, but body condition is only related to color characteristics of the red postorbital patch and the H/L ratio is only related to coloration of the yellow chin strips. This suggests that these color traits may signal different, and probably complementary, information on the general and current health state of an individual. It is likely that "red" and "yellow" pigments have different physiological trade-offs. Each color component might not reflect the same information because it is not produced through the same metabolic processes and thus, should not be perceived similarly by conspecifics. Further studies should examine the pigments responsible of these colorations and how they can be affected by the physiological health state of a turtle.

The role of these colorful patches and stripes as visual sexual signals is very likely. On the one hand, in the eyes of freshwater turtles, several cone visual pigments absorb maximally, among others, around 530 nm and 620 nm wavelengths (Ohtsuka 1985; Loew and Govardovskii 2001), which coincides very well with the wavelengths with maximum reflectance of the head color patches measured in this study. This is probably explained by the same pigments found in colorful cone oil droplets and those used in skin stripes, as it occurs in birds (Toomey and McGraw 2009). Sensory systems are traditionally considered to be optimized within a given species to detect specific important stimuli (e.g., prey and mates) under particular environmental conditions (Endler 1992; Endler and Basolo 1998; Bradbury and Vehrencamp 2011). Therefore, since the eyes of these turtles seem especially sensitive to the colors that are present in the head, we could expect that these colors are important in intraspecific communication. On the other hand, the courtship of this turtle includes visual displays in which these color patches are shown to prospective mates (Jackson and Davis 1972). Interestingly, ontogenetic melanization eventually obscures the color pattern and results in an almost completely black appearance of the shell and skin of older males (Lovich et al. 1990a), and melanistic males have courtship that involves less visual displays, such as titillation, and more chasing and biting behaviors (Thomas 2002). This might suggest that once color patches are not effective in communicating the quality of a male to females, inducing them to mating, more active strategies are needed to attempt or force copulation. Further studies should manipulate the characteristics of coloration of head stripes of turtles examining their effects on courtship success.

The color patches of *T. scripta* are present in males and females and there are no intersexual differences in characteristics of coloration, although the red patch may be smaller in old males. This suggests that color patches may function as signals in both sexes. On the one hand, males may advertise their health state to females during courtship, which is highly stereotyped (Jackson and Davis 1972; Thomas 2002). Similarly, coloration of female *T. scripta elegans* might advertise reproductive quality to prospective males.

In fact, female of other subspecies *T. scripta scripta* show an active role during courtship with similar display movements to that of males (Lovich et al. 1990b). Female lizards *Sceloporus virgatus* develop pterin-based orange color patches during the reproductive season that may influence male behavior. This coloration may advertise egg quality because more ornamented females may produce higher quality offspring, in part, because their eggs contain more antioxidants (Weiss et al. 2011). Male turtles might select females based on female coloration signaling health state and body condition, which will affect the quality of eggs and the offspring. In the Spanish terrapin, *M. leprosa* males select water used by females with a better immune response, suggesting that males prefer to mate with these healthier females (Ibáñez et al. 2012).

Coloration of male turtles might also be used in agonistic contests with other males as it occurs in many lizards where characteristics of coloration may signal fighting ability (Anderholm et al. 2004; Martín and López 2009), which may depend on the health state and body condition of a male. This information may allow conspecific males to assess with reliability the fighting ability of a male at a long distance and decide whether to engage in an agonistic contest or avoid the fight. In addition, in turtles, this information might be useful not only during the mating season, and not only between males, but also between males and females, during competition for basking places (Polo-Cavia et al. 2010b). In this sense, the presence of colorful skin patches in juvenile turtles, too, could be used in signaling during intraspecific competition.

We conclude that the allocation of pigments, probably carotenoids, to head skin coloration of *T. scripta elegans* is probably costly because there seem to be a trade-off with the role of these pigments in the maintenance of the immune function. Only healthier turtles might be able to develop their coloration with certain characteristics. This trade-off would confer honesty to coloration, which could be used in intraspecific communication and sexual selection processes. Many other turtle species, especially aquatic turtles, have similar colorful patches (Ernst and Barbour 1989), so it is very likely that similar relationships between health and coloration are widespread among freshwater turtles. Therefore, color patterns of the head and other skin areas may have a still unexplored significant role in sexual selection in many turtles, which merits further studies.

Ethical standards Experiments complied with all current laws of Spain and the Environmental Agency (Consejería de Medio Ambiente) of the Comunidad de Madrid Local Government where they were carried out.

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