

Pterin-based ornamental coloration predicts yolk antioxidant levels in female striped plateau lizards (*Sceloporus virgatus*)

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Summary

1. Maternal investment in egg quality can have important consequences for offspring fitness. For example, yolk antioxidants can affect embryonic development as well as juvenile and adult phenotype. Thus, females may be selected to advertise their yolk antioxidant deposition to discriminatory males via ornamental signals, perhaps depending on the reproductive costs associated with signal production.

2. Female striped plateau lizards (*Sceloporus virgatus*) develop pterin-based orange colour patches during the reproductive season that influence male behaviour and that are positively associated with the phenotypic quality of the female and her offspring. Here, we assessed one potential developmental mechanism underlying the relationship between offspring quality and female ornamentation in *S. virgatus*, by examining the relationship between ornament expression and yolk antioxidant levels.

3. As expected, concentrations of the yolk antioxidants vitamin A, vitamin E and carotenoids (lutein and zeaxanthin) were strongly positively intercorrelated. Eggs from larger clutches had fewer antioxidants than eggs from smaller clutches, suggesting that females may be limited in antioxidant availability or use. Fertilized and unfertilized eggs did not differ in yolk antioxidant levels.

4. The size of a female's ornament was positively related to both the concentration and total amount of yolk antioxidants, and ornament colour was positively related to yolk antioxidant concentration. Thus, in *S. virgatus*, female ornaments may advertise egg quality. In addition, these data suggest that more ornamented females may produce higher-quality offspring, in part because their eggs contain more antioxidants. As the colour ornament of interest is derived from pterins, not carotenoids, direct resource trade-offs between ornaments and eggs may be eliminated, reducing reproductive costs associated with signalling.

5. This is the first example of a positive relationship between female ornamentation and yolk antioxidants in reptiles and may indicate the general importance of these patterns in oviparous vertebrates.

Key-words: indicator mechanism, investment hypothesis, maternal effects, nuptial colour

Introduction

A female can influence the quality of her offspring through both genetic and nongenetic (environmental) mechanisms. An important nongenetic mechanism involves the allocation of nutrients and other biochemicals to the embryo via placental transfer or yolk. Egg yolk, which consists primarily of protein and lipids (Thompson & Speake 2003), may vary both within and among clutches in the concentrations and

amounts of important biochemicals such as fatty acids, antibodies, steroid hormones and antioxidants (Schwabl 1996; Wiegand 1996; Speake *et al.* 1999; Gasparini *et al.* 2001; Izquierdo, Fernández-Palacios & Tacon 2001; Boulinier & Staszewski 2008). These biochemicals, as well as egg size itself, may influence embryonic development, neonatal growth and survival (e.g., George *et al.* 2001; Izquierdo, Fernández-Palacios & Tacon 2001; Meylan & Clobert 2005; Johnston *et al.* 2007; Wagner & Williams 2007; Boulinier & Staszewski 2008). Thus, how a female nourishes her egg yolk may have important fitness consequences for her offspring.

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Yolk antioxidants have been proposed to be particularly important to offspring health, as rapid embryonic growth rates result in relatively high levels of oxidative stress (Blount, Houston & Møller 2000; Surai, Speake & Sparks 2001). Normal cell processes within the body, such as mitosis and respiration, produce reactive chemical species (i.e., free radicals) that can damage DNA, protein, lipids and other molecules, thereby impairing the function of multiple chemical systems and causing oxidative stress. As a consequence of oxidative stress, important physiological processes can be compromised and pathologies can develop (Valko *et al.* 2007). Antioxidants, such as some vitamins (e.g., tocopherol) and carotenoids, can quench reactive chemical species before damage to the body occurs (Krinsky 2001). As proper cell proliferation and tissue growth are essential to developing animals, increased investment in egg antioxidants has been found to promote offspring size, growth rates, pigmentation, immunity, resistance to oxidative stress and survival in invertebrates, fishes and birds (e.g., Surai, Noble & Speake 1999; George *et al.* 2001; Saino *et al.* 2003; McGraw, Adkins-Regan & Parker 2005; Bazzyar Lakeh *et al.* 2010; but see Grether *et al.* 2008).

Given the apparent importance of antioxidants to offspring fitness, we might expect to see little variation in the amount deposited in yolk; however, a number of studies have found significant variation in yolk antioxidant deposition both within and among females (Royle *et al.* 1999; Royle, Surai & Hartley 2001; Bortolotti *et al.* 2003; Navara *et al.* 2006; Safran *et al.* 2008). This pattern suggests that there are costs to egg antioxidant deposition and that males could benefit from assessing potential female mates for their differential ability to meet those costs. Thus, there may be selection on females to communicate their egg quality via a

condition-dependent ornament (Blount, Houston & Møller 2000), the strength of which may depend on the costs associated with signal production and egg production (Fitzpatrick, Berglund & Rosenqvist 1995). While female ornaments were traditionally considered to be functionless by products of sexual selection pressures on conspecific males (Lande 1980), recent work suggests that they are often functionally significant by advertising aspects of female quality to conspecifics (Amundsen 2000; Kraaijeveld, Kraaijeveld-Smit & Komdeur 2007; Clutton-Brock 2009). Perhaps the strongest case for direct selection on female ornaments occurs in instances with female-specific ornamentation (Amundsen & Forsgren 2001; Domb & Pagel 2001; Massironi, Rasotto & Mazzoldi 2005; Weiss 2006).

Here, our aim was to investigate whether female-specific ornaments may advertise information about maternal deposition of yolk antioxidants. To date, only a small number of studies have examined the relationship between female ornamentation and yolk antioxidants (Table 1), and only one has focused on a female-specific ornament (Svensson *et al.* 2006). Female two-spotted gobies (*Gobiusculus flavescens*) develop nuptial belly colour because, in part, of their eggs being directly visible through translucent skin (Svensson *et al.* 2005), and females with more colourful bellies produce eggs with higher concentrations of antioxidants (Svensson *et al.* 2006). In lesser black-backed gulls (*Larus fuscus*; Blount *et al.* 2002) and zebra finches (*Taeniopygia guttata*; McGraw, Adkins-Regan & Parker 2005), females fed a high-carotenoid diet were more colourful and deposited more antioxidants into egg yolk than control females; more colourful females tended to deposit more antioxidants in yolk (McGraw, Adkins-Regan & Parker 2005) or had a smaller decline in yolk antioxidants with lay order (Blount *et al.* 2002). In these

Table 1. Summary of studies examining female ornament–yolk antioxidant relationships, including relevant characteristics of the female ornament (the trait, its relative expression in males vs. females and the dominant pigment type). ‘Male-typical’ traits are more elaborately expressed by males than by females, ‘monomorphic’ traits are nearly equally expressed by the two sexes, and ‘female-specific’ traits are only expressed by females.

Taxonomic group	Female trait	Trait type	Pigment	Relationship to yolk antioxidants	Reference
Birds					
Lesser black-backed gull	Integument colour	Monomorphic	Carotenoid	Positive ^b	Blount <i>et al.</i> (2002) ^a
Zebra finch	Bill colour	Male-typical	Carotenoid	Positive	McGraw, Adkins-Regan & Parker (2005) ^a
Blue tit	Breast plumage colour	Male-typical	Carotenoid	None	Biard, Surai & Møller (2005) ^a
Barn swallow	Belly plumage colour	Male-typical	Melanin	Negative	Safran <i>et al.</i> (2008)
	Tail streamer length			Negative	
Fish					
Three-spined stickleback	Pelvic spine colour	Monomorphic	Carotenoid	Negative ^c	Nordeide, Rudolfson & Egeland (2006)
Arctic charr	Belly and fin colour	Male-typical	Carotenoid	None	Nordeide <i>et al.</i> (2008)
Two-spotted goby	Belly colour	Female-specific	Carotenoid	Positive	Svensson <i>et al.</i> (2006)
Lizard					
Striped plateau lizard	Throat patch colour	Female-specific	Pterin	Positive	Present study
	Throat patch size			Positive	

Studies marked with ^aused diet manipulation, whereas others explored natural variation; ^bEgg antioxidants were not directly related to female colour but were related to a female colour X lay order interaction; ^cMales prefer drabber females (Nordeide 2002).

cases, females may signal their reproductive quality to males, and males may be selected to prefer more ornamented females as mates (e.g., Amundsen & Forsgren 2001). In contrast, more colourful female three-spined sticklebacks (*Gasterosteus aculeatus* L.) deposit fewer antioxidants in their yolk (Nordeide, Rudolfsen & Egeland 2006) and males show a mate preference for drabber females (Nordeide 2002), again indicating the signal capabilities of female ornaments, though suggesting an alternative function for the expression of female colour in this species.

While previous studies have focused on birds and fishes (Table 1), lizards make excellent subjects for such work. Firstly, parental care to offspring stops at the point of egg-laying in most oviparous lizards; in these cases, all resources provided to offspring are packaged within the egg, making maternal yolk provisioning especially important to hatchling development. Secondly, Blount, Houston & Møller (2000) suggest that the use of signals to advertise yolk investment may be strongest in [bird] species with relatively large clutch mass to body mass ratios. Relative clutch mass (RCM) is a well-studied life-history characteristic in lizards (Vitt & Congdon 1978), where sit-and-wait foragers are known to have much higher RCM values than wide-ranging foragers. In addition, whereas birds yolk and lay eggs of a given clutch sequentially (e.g., lay one egg a day throughout a laying period), most lizard species produce the eggs of a clutch simultaneously; this could potentially exaggerate the effect of RCM on antioxidant allocation trade-offs. Thirdly, it is often easy to distinguish fertilized and unfertilized lizard eggs by visual inspection (based on the presence or absence of a vascularized embryonic disc), allowing for fertilization status to be considered in analyses. As fertilization occurs between ovulation and oviposition, an effect of fertilization status on yolk antioxidants would suggest a high degree of active female control. Finally, over thirty species of lizards express female-specific ornamentation, comprised of yellow, orange or red patches of pigmentation that develop in concert with the female reproductive cycle (Cooper & Greenberg 1992).

Female striped plateau lizards (*Sceloporus virgatus*) are oviparous, sit-and-wait foraging animals with one of the highest RCM values documented for lizards (mean clutch mass/body mass = 0.340; Vitt & Congdon 1978). They also develop orange, pterin-based pigmentation on the underside of their throats during vitellogenesis (Weiss 2002; S. L. Weiss and J. Hudon, unpublished data). Previously, we found that social behaviour in males is responsive to variation in female ornamental coloration (Weiss 2002), that both the colour and the size of the ornament are positively related to indices of female phenotypic quality (Weiss 2006) and that the size of a female's ornament is positively related to the phenotypic quality of her offspring (Weiss, Kennedy & Bernhard 2009). We hypothesized that one mechanism by which more ornamented females produce higher-quality offspring may be increased deposition of yolk antioxidants. To examine this question, we first determined the degree of variation in yolk antioxidants within and among females and then determined whether natural among-female variation in yolk antioxidants

was explained by ornament size and colour of mothers. If female phenotype is an indicator of egg quality, we predicted that the size or colour of female ornamentation would be positively correlated with yolk antioxidant concentrations and/or total amounts (Blount, Houston & Møller 2000).

Materials and methods

ANIMAL MAINTENANCE

We collected by noose 20 free-ranging *S. virgatus* females (mean \pm SE snout-to-vent length (SVL): 62.5 ± 0.6 mm; body mass: 7.58 ± 0.22 g) on 16–18 May 2006 by searching species-typical habitat along the north fork of Cave Creek and surrounding plateaus within 3.5 km of the American Museum of Natural History's Southwestern Research Station (SWRS), Portal, AZ, USA. Of all adult females observed during our collecting effort, 67% were haphazardly assigned to this study. At SWRS, females were randomly assigned to one of two outdoor enclosures ($4.3 \times 1.7 \times 2.2$ m; 10 females per enclosure). Each enclosure contained a soil substrate and several rocks, logs and bricks for perches and shelter. Each enclosure also contained five randomly assigned wild-caught males (SVL: 56.1 ± 1.0 mm; body mass: 5.08 ± 0.21 g) to allow for courtship and mating. Animals were toe-clipped for individual identification and provided water *ad libitum*. About 30 crickets (*Acheta* sp.) were added to each enclosure every other day. When all females were gravid and their reproductive colour expression had peaked, males were released at their capture site and females were transported to the University of Puget Sound (UPS), Tacoma, WA, USA, over a 3-day period in individual cloth bags inside a chilled cooler.

Sceloporus virgatus females oviposit at the onset of the summer rainy season, typically in early July, but do not oviposit naturally in captivity (Weiss, Jennings & Moore 2002). Thus, at UPS, females were induced to oviposit their clutch via intraperitoneal injections of 2 USP units of oxytocin in 0.1 mL of aqueous solution. Six females laid partial clutches, so we obtained their last eggs by dissection ($n = 8$ dissected eggs; 4.5% of all eggs in sample); whether an egg was laid or dissected had no effect on antioxidant concentration ($F_{1,158} = 0.25$, $P = 0.62$) or total antioxidant amount ($F_{1,155} = 2.61$, $P = 0.11$). All eggs were oviposited or dissected 6–12 July 2006. Each egg was categorized as fertilized or unfertilized and weighed intact; the shell was then split and egg contents were transferred to an Eppendorf tube for storage at -70 °C until extraction. Egg contents were primarily yolk, but also included extra-embryonic membranes and embryonic tissue (when fertilized).

MEASURING MATERNAL CHARACTERISTICS

The reproductive coloration of *S. virgatus* females develops over the course of several weeks, and colour patches reach peak intensity and size around the time of ovulation (Weiss 2002). We wanted to use the characteristics of each female's peak ornament expression for analyses. Thus, we tracked female colour development and determined peak expression as follows. Every 2 days from 16 May to 27 June 2006, we compared each female's developing colour patch to Munsell Color Chips (Munsell/MacBeth/Kollmorgen Corporation, Baltimore, MD, USA) to determine ornament brightness (i.e., Munsell 'value') and saturation (i.e., Munsell 'chroma'). As there is little variation in the hue of the ornament, all females were matched to Munsell hue 10R and hue is not considered in analyses. To standardize measurements, EAK performed all colour matches under the

same light conditions (Pelican Super SabreLite). On the same days, we tracked female ornament size by photographing the right colour patch of every female (along with a calibration scale) using an Olympus Camedia 5.0 mega-pixel digital camera (Olympus, Center Valley, PA, USA), and we measured SVL and body mass. Two people (E.A. Kennedy and P. E. Michael) independently viewed each individual's series of photographs to visually determine the date on which that individual's ornament reached peak expression. In cases of disagreement ($n = 2$), a third viewer (SLW) independently analysed the photo series, and peak colour expression was determined to occur on the date that had the most agreement between viewers.

We imported digital photographs to a computer at a resolution of 2560×1920 pixels and analysed peak-expression photos for ornament size using an automated 'select colour' command in Adobe Photoshop CS2 9.0 that standardizes the colour shade selection. We calculated the area of the selected region in mm^2 , hereafter called 'orange area,' using Scion Image release Alpha 4.0.3.2 (Scion Corporation, Frederick, MD, USA). Other maternal characteristics on the day of peak colour expression were also used in statistical analyses, including a principle component score (PC1) based on Munsell value and chroma measurements that explained 71% of variation in ornament colour (hereafter called 'colour'), body mass, SVL and body condition (standardized residuals from a regression of body mass on SVL³; Weiss 2006). The use of alternative indices of condition (e.g., residuals from a regression of body mass on SVL, or simple body mass:SVL ratios) did not affect our conclusions (data not shown).

YOLK ANTIOXIDANT ANALYSES

Egg contents were thawed and homogenized prior to extraction. Lipid-soluble antioxidants were extracted from each egg using a typical organic solvent procedure. We combined an aliquot of the homogenate (0.092–0.212 g) with 1 mL of deionized water, 1 mL of 70% ethanol and 1 mL of hexane:methyl *tert*-butyl ether (MTBE) (1:1 v/v), vortexed for 5 min, centrifuged for 5 min at 3000 r.p.m. and collected the resulting supernatant. The extraction was repeated with 1 mL 1:1 hexane:MTBE and 100 μL of a high-concentration NaCl solution (to prevent gelling). Extracts were evaporated to dryness under nitrogen gas, and the residue was frozen and shipped under nitrogen gas on dry ice to Arizona State University (ASU), Tempe, AZ, USA.

At ASU, we analysed yolk antioxidants via high-performance liquid chromatography (HPLC) (as described by McGraw, Adkins-Regan & Parker 2002). Based on preliminary analyses of *S. virgatus* yolk antioxidant composition performed by Craft Technologies (Wilson, NC, USA), we focused our HPLC analyses on vitamin A (retinol), vitamin E (tocopherol) and the following carotenoids: *trans*-lutein, *cis*-lutein isomers and *trans*-zeaxanthin; other carotenoids such as cryptoxanthin, lycopene and carotene were undetected in these preliminary assays. We redissolved the residue in 200 μL of HPLC mobile phase (methanol–acetonitrile–chloroform, 46:46:8, v/v/v) and injected 50 μL into a WatersTM 717plus Autosampler HPLC (Millipore Corp., Milford, MA, USA) fitted with a Develosil RPAqueous RP-30 HPLC column (250×4.6 mm I.D.; Nomura Chemical Co., Ltd., Aichi, Japan) and ran an isocratic system (HP 1050 Series Isocratic Pump; Hewlett-Packard, Palo Alto, CA, USA) of the aforementioned mobile phase for 12.5 min at a constant flow rate of 1.2 mL min^{-1} . We confirmed the identity of yolk antioxidants by comparing retention times and λ_{max} values to those for authentic reference vitamins and carotenoids that we ran as external standards. Light absorbance data were collected from 290 to 550 nm using a Waters 2996 photodiode array detector (Milford, MA, USA).

STATISTICAL ANALYSES

We applied log transformations to normalize the distributions of all yolk antioxidants and orange area. We used smoothing splines to graphically characterize the relationships between continuous variables; these were only used to characterize the relationship between variables of interest (e.g., whether they were linear or not) so that appropriate models could be constructed (e.g., whether higher-order terms were necessary in linear models). We constructed very conservative splines using a flexible tuning parameter ($\lambda = 1$) to construct pictorial relationships between variables of interest.

We present results based on concentration (μg antioxidant per g homogenate) and on total amount (μg antioxidant per whole egg), as it is unclear which is more relevant to developing *S. virgatus* embryos and so that patterns concerning similarities and differences in concentration and amount data can continue to accumulate across species (McGraw 2005; Safran *et al.* 2008). Unless otherwise stated, data were analysed using general linear models, with 'mother ID' as a random effect to control for the lack of independence of eggs laid by the same mother. We included 'clutch size' as a covariate in our models to control for possibly confounding effects of this variable. We used the Satterthwaite method to estimate the denominator degrees of freedom, which performs well with unbalanced data (Littell *et al.* 2002), or in our case, unequal clutch sizes. All analyses were performed using SAS v. 9.2 (Cary, NC, USA).

Results

CORRELATIONS AMONG ANTIOXIDANTS

In *S. virgatus* eggs, vitamin E was the most abundant antioxidant (Fig. 1), followed by *trans*-lutein, vitamin A, *trans*-zeaxanthin and *cis*-lutein. Within eggs, concentrations and amounts of the five antioxidants were all significantly positively intercorrelated (all coefficients > 0.66 , $P < 0.001$). Controlling for the lack of independence among eggs from the same mother, we found that clutch size did not significantly affect the intercorrelations of vitamins and carotenoids in any model; removing clutch size from the models did not alter the overall interpretation. Thus, all subsequent analyses are performed on summed variables, hereafter called 'antioxidant concentration' and 'total antioxidant amount'.

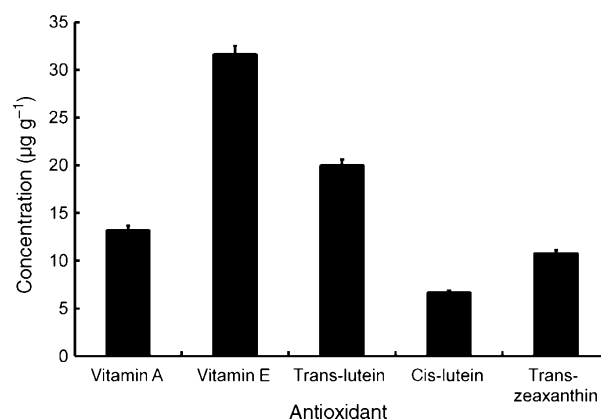


Fig. 1. Mean (\pm SE) concentration ($\mu\text{g g}^{-1}$ egg mass) of antioxidants in the eggs ($n = 175$) of *Sceloporus virgatus* females.

EFFECT OF MOTHER ID AND CLUTCH CHARACTERISTICS ON YOLK ANTIOXIDANTS

Using both 'mother ID' and 'clutch size' as covariates in a single model, we found that antioxidant concentration decreased as clutch size increased (coefficient estimate \pm SE = -0.0204 ± 0.0078 ; $F_{1,172} = 6.9$, $P < 0.01$) and that significant among-female variation in antioxidant concentration remained while controlling for the effect of clutch size (coefficient = 0.0002 ± 0.00005 ; $F_{1,172} = 16.95$, $P < 0.0001$; full model-adjusted $R^2 = 0.09$). We also found a significant negative relationship between total yolk antioxidant amount and clutch size (coefficient = -0.0171 ± 0.0075 ; $F_{1,169} = 5.2$, $P = 0.02$) that could effectively explain among-female variation in total antioxidant amount (coefficient = 0.00001 ± 0.00005 ; $F_{1,169} = 0.04$, $P = 0.85$; full model-adjusted $R^2 = 0.03$). Egg mass may also explain among-female variation in yolk antioxidants. However, because egg mass and clutch size were negatively correlated (bivariate correlation: $r = -0.19$, $P = 0.01$, $n = 176$), we chose to exclude egg mass and use only clutch size as a covariate in our analyses. Fertilization status did not significantly relate to either yolk antioxidant concentration (coefficient = 0.0077 ± 0.0403 ; $F_{1,170} = 0.04$, $P = 0.85$) or amount (coefficient = -4.7600 ± 2.5401 ; $F_{1,165} = 3.51$, $P = 0.06$; Table S1).

EFFECT OF MATERNAL PHENOTYPE

To avoid the issues of multicollinearity and to determine which maternal phenotype characteristics were important to include in our final model, we first searched for bivariate correlations among our independent variables: maternal body mass, body size (SVL), body condition, ornament size (orange area) and ornament PC score (colour). The only significant relationships found were between body mass and SVL ($r = 0.63$, $P = 0.005$, $n = 18$), and body mass and body condition ($r = 0.79$, $P = 0.0001$, $n = 18$). Thus, body mass alone could effectively control for mothers' body shape and was used as the sole covariate to explore body shape relationships in our models. Body mass, orange area and colour were uncorrelated (r range: -0.12 to 0.11 ; all $P > 0.64$); these three variables were therefore included in the final model along with mother ID (as a random effect) and clutch size.

When controlling for mother ID and clutch size, egg antioxidant concentration was positively related to both ornament size and colour (Fig. 2a–c; orange area: coefficient = 0.1902 ± 0.0427 , $F_{1,170} = 19.9$, $P < 0.0001$; colour: coefficient = 0.0225 ± 0.0100 , $F_{1,170} = 5.1$, $P = 0.03$) and was not related to female body mass (coefficient = 0.0085 ± 0.0130 , $F_{1,170} = 0.4$, $P = 0.51$; full model-adjusted $R^2 = 0.15$; Table S2). Total antioxidant amount was positively related to female ornament size and body mass and was not related to ornament colour (Fig. 2d–f; orange area: coefficient = 0.1659 ± 0.0380 , $F_{1,167} = 19.1$, $P < 0.0001$; colour: coefficient = 0.0058 ± 0.0089 , $F_{1,167} = 0.4$, $P =$

0.52 ; body mass: coefficient = 0.0400 ± 0.0116 ; $F_{1,167} = 11.9$, $P = 0.0007$; full model-adjusted $R^2 = 0.18$; Table S2).

Discussion

We show, for the first time in lizards, that female ornamentation honestly reflects the patterns of antioxidant deposition to eggs and may serve as a signal of egg quality to potential mates. The availability of antioxidants to developing embryos can have significant consequences for offspring fitness in other oviparous species (Surai, Noble & Speake 1999; George *et al.* 2001; Saino *et al.* 2003; McGraw, Adkins-Regan & Parker 2005) and thus may be one mechanism underlying our previously established relationship between female ornament size and offspring characteristics (body condition and sprint speed) in *S. virgatus* (Weiss, Kennedy & Bernhard 2009). However, the effect of vitamin E, vitamin A and carotenoid concentrations/amounts on the development of this and other reptile species is relatively unexplored and should be directly assessed. Like previous studies examining maternal investment to yolk (Pilz *et al.* 2003; Safran *et al.* 2008), our results for concentration and for total amount of yolk antioxidants differed somewhat. When statistically controlling for maternal identification and clutch size, both measures of yolk antioxidants were positively related to ornament size; however, antioxidant concentration was also positively related to ornament colour, whereas total antioxidant amount was positively related to maternal body mass. It is currently unknown which measure of antioxidant deposition may be more biologically relevant to embryonic development or how variation in either measure affects embryonic yolk utilization. Regardless, as *S. virgatus* females with larger and darker patches are themselves of higher quality (Weiss 2006), these data are consistent with the investment hypothesis, which predicts that high-quality females (and females mated to high-quality males) invest more resources into eggs than do low-quality females (Gil *et al.* 1999; Safran *et al.* 2008).

The concentration of carotenoids and vitamins that we detected in *S. virgatus* eggs was similar to (within an order of magnitude of) that detected in other oviparous reptiles (Speake, Surai & Gore 2001; Dierenfeld *et al.* 2002) and birds (e.g., Royle *et al.* 1999; Biard, Surai & Møller 2005; Navara *et al.* 2006), with the exception of extremely low vitamin A levels in the eggs of Hermann's tortoises (*Testudo hermanni boettgeri*; Speake, Surai & Gore 2001). Vitamin E is consistently the most abundant antioxidant detected in yolk of these species and is known to play a major role in protecting developing tissues from oxidative damage (e.g., Surai, Noble & Speake 1999; Blount, Houston & Møller 2000). Among *S. virgatus* females, the concentration and total amount of antioxidants per egg were negatively correlated with clutch size. This relationship may reflect within-individual dietary limitation of antioxidants and/or a physiological trade-off of antioxidants to reproduction vs. health (Blount *et al.* 2004). High reproductive effort (such as a large clutch size) can increase a female's susceptibility to oxidative stress (reviewed

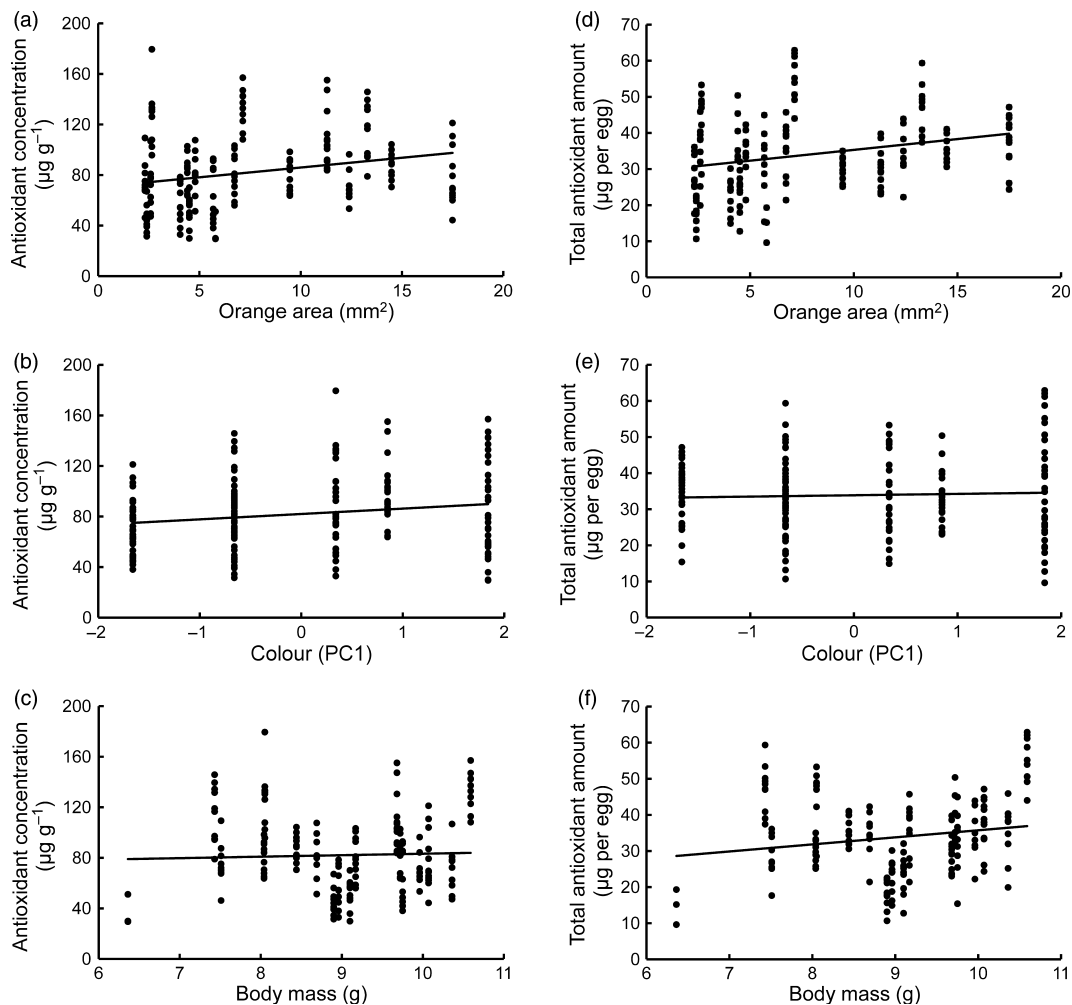


Fig. 2. Relationships between female ornament size (orange area), colour (PC1 score) and body mass with antioxidant concentration (a–c) and total antioxidant amount (d–f) in the eggs of *Sceloporus virgatus* females. Vertical groupings represent eggs from one or a few females.

by Dowling & Simmons 2009), increasing the need to utilize antioxidants for the maintenance of her own health and thereby decreasing the amount available to deposit in yolking follicles. Variation in female ability to withstand such trade-offs may be linked to their ability to obtain and extract antioxidants from their diet (Endler 1980) or their current disease state (Lozano 1994).

Blount, Houston & Møller (2000) first hypothesized that positive relationships between yolk antioxidant levels and female ornamentation may be expected among oviparous species, given the importance of antioxidants to both mothers and their developing offspring. Since then, such relationships have been found in a few bird and fish species (McGraw, Adkins-Regan & Parker 2005; Svensson *et al.* 2006) and now in lizards. One particular class of antioxidant – carotenoids – has been particularly well examined for its role in maintaining honesty of sexual signals (e.g., Endler 1980; Kodric-Brown 1989; Lozano 1994; Olson & Owens 1998), as it is a common basis for yellow, orange and red ornaments found in nature; this emphasis is evident within the work conducted

thus far on the deposition of antioxidants by females to ornaments and yolk (Table 1). Although the expression of carotenoid-based signals has long been assumed to reflect a direct trade-off in usage of carotenoids (e.g., Lozano 1994), recent work suggests that carotenoids may play a relatively minor antioxidant role (Costantini & Møller 2008; Olsson *et al.* 2008a). Thus, the signals may instead represent an indirect trade-off influenced by various antioxidant interactions, such as the ability of noncarotenoid antioxidants (e.g., vitamin E) to protect carotenoids from oxidation and bleaching (Hartley & Kennedy 2004; Catoni, Peters & Schaefer 2008; Biard *et al.* 2009). An interesting alternative occurs in two-spotted gobies (*Gobiusculus flavescens*), where carotenoid deposition to eggs directly and positively enhances the female ornament, as the eggs themselves are visible through the female's translucent belly skin (Svensson *et al.* 2006).

There are other biological pigments than carotenoids that exhibit antioxidant properties (McGraw 2005) and thus may also serve as honest signals of antioxidant deposition to yolk. Among some reptiles, including *S. virgatus*, carotenoids play

a relatively minor role in integument pigmentation, and in these cases, orange and red ornamental signals are instead primarily produced with pterins (Ortiz *et al.* 1963; Ortiz & Maldonado 1966; Cooper & Greenberg 1992; S. L. Weiss and J. Hudon, unpublished data). Pterins are synthesized via purine salvage pathways (Morrison, Rand & Frost-Mason 1995) and can act as essential cofactors in enzymatic reactions (Oettl & Reibnegger 2002). Activation of the immune system may trigger pterin release from immune cells; in fact, pterin levels may be used in clinical settings to monitor disease progress (Huber *et al.* 1984). Pterins are known to reduce oxidative stress both indirectly and directly by scavenging free radicals, but may also act as pro-oxidants under certain laboratory conditions (reviewed by Oettl & Reibnegger 2002). To date, these processes have not been tested in wild animal populations and there is no work directly examining potential trade-offs in pterin allocation to immune response and ornamentation.

In *S. virgatus* females, it is the extent of pterin ornamentation that honestly reflects the deposition of carotenoids and lipid-soluble vitamins in yolk. Because different biomolecules are utilized in the colour signal vs. in egg yolk, females do not directly trade-off carotenoid allocation to ornaments vs. reproduction in this species. The presence of direct resource trade-offs may limit the evolution and exaggeration of female signals of mate quality (and male mate preferences) because of high reproduction costs, while, in contrast, their absence may relax counterselection on the female ornament (Fitzpatrick, Berglund & Rosenqvist 1995). Any trade-off between ornament and offspring production in female *S. virgatus* is hypothesized to be indirect via the availability and demand of various interacting antioxidant molecules and the ability of the female to withstand the costs of oxidative stress (Catoni, Peters & Schaefer 2008; Dowling & Simmons 2009). Experiments are needed to determine whether females are faced with an internal trade-off in pterin allocation (to ornaments vs. health) and in carotenoid/vitamin allocation (to health vs. egg quality) and how these resource trade-offs may interact with each other and with female condition. It may be predicted that oxidative stress reduces both ornament expression and egg quality of *S. virgatus* females. In the polymorphic painted dragon (*Ctenophorus pictus*), males with higher oxidative stress, as measured by basal superoxide levels, had reduced development of head coloration relative to males with lower oxidative stress (Olsson *et al.* 2008a); interestingly, carotenoid intake did not affect this relationship among males with either carotenoid-based yellow heads or noncarotenoid-based (likely pterin-based) red heads (Olsson *et al.* 2008a). In some other reptilian species, orange and red colour appears to be based on a composite of pterins and carotenoids (Steffen & McGraw 2007). This variety both within and among species in the biochemical basis of ornamental colour allows for potentially interesting comparisons among lizards.

To our knowledge, only one other study has searched for a relationship between female ornament expression and yolk antioxidant deposition for noncarotenoid-based ornaments

(Table 1); in North American populations of barn swallows, melanin-based belly plumage of females was negatively related to yolk antioxidant deposition (Safran *et al.* 2008). The investment hypothesis predicts that high-quality females invest more in reproduction than do low-quality females (Pilz *et al.* 2003; Safran *et al.* 2008); if one assumes that more ornamented females are higher quality than less ornamented females, then negative relationships between female ornaments and yolk antioxidant deposition appear contradictory to the hypothesis. Such negative relationships may be explained by unmet assumptions (Nordeide, Rudolfson & Egeland 2006) or by within- or between-season effects (Safran *et al.* 2008). Between-season effects may be particularly relevant in studies focused on plumage ornaments (Biard, Surai & Møller 2005; Safran *et al.* 2008), as antioxidant availability and physiological demand may vary significantly between the periods of moult and reproduction (Biard, Surai & Møller 2005), which could be separated by many months. In contrast, *S. virgatus* females develop ornamentation and yolking follicles simultaneously (Weiss 2002).

Nordeide, Rudolfson & Egeland (2006) suggest that female ornament–yolk antioxidant relationships may vary as a function of degree of sexual dichromatism (i.e., female-specific ornaments or ornaments also expressed in males). Although examples to date are still limited, no clear pattern has yet emerged in this regard, as positive relationships between ornamentation and yolk investment have been found in species with all forms of female ornaments (Table 1). Presumably, in these cases, females benefit by honestly signalling their investment of antioxidants to eggs (i.e., their egg quality) to potential mates and males respond appropriately. The reduction of reproductive cost associated with female ornamentation, which in *S. virgatus* may be mediated by utilizing different biomolecules for ornaments and egg quality, may be critical to the evolution of both honest female signals of mate quality and the male response (Fitzpatrick, Berglund & Rosenqvist 1995). Thus, future efforts should also aim to assess whether female ornament–yolk antioxidant relationships vary as a function of pigment type.

In *S. virgatus*, males are known to preferentially respond during the courtship period to females with darker orange ornamentation (Weiss 2002); though, male response to ornament size has not yet been empirically determined. The short, synchronous reproductive season of *S. virgatus* (Weiss 2002) may add selective pressure on males to be discriminatory in the allocation of their own reproductive effort to available females, as time limitations are expected to reduce the levels of polygamy (Emlen & Oring 1977; Dewsbury 1982; Langmore *et al.* 1996). By selecting more ornamented females, males may benefit by producing higher-quality offspring. This effect could occur by siring offspring with higher-quality developmental environments (i.e., higher yolk antioxidant concentration or amounts), but also by siring offspring who receive high-quality maternal genes for resistance to oxidative stress (von Schantz *et al.* 1999; Olsson *et al.* 2008b). *S. virgatus* females may benefit from advertising their reproductive quality by attracting males into their home range,

reducing energy expended on mate searching and supporting a sedentary lifestyle that increases their investment in their single annual clutch (Rose 1981; Olsson & Madsen 1995), while gaining access to multiple male partners to promote male–male (direct or sperm) competition and reduce the risk of genetic incompatibility (Madsen *et al.* 1992).

In conclusion, we found evidence consistent with the hypothesis that antioxidants are environmentally and/or physiologically limiting to *S. virgatus* females and that those females that invest more antioxidants towards reproduction also develop larger pterin-based female-specific ornaments. Females with larger ornaments tend to produce higher-quality offspring (Weiss, Kennedy & Bernhard 2009), and yolk antioxidant deposition may be one maternal effect underlying this relationship. Future work should directly test the effect of yolk antioxidants on *S. virgatus* offspring condition. Antioxidant concentration, but not total antioxidant amount, was also positively related to ornament colour, whereas total antioxidant amount was positively related to female body mass. As recent work has called into question the direct antioxidant role of carotenoids (Costantini & Møller 2008; Olsson *et al.* 2008a), we encourage more work to investigate noncarotenoid traits for similar relationships, as well as to directly assess the antioxidant function of carotenoids in lizards.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Test statistics from a general linear model with egg fertilization status as the independent variable.

Table S2. Test statistics from a general linear model with maternal ornament characteristics (orange area and colour PC) and body mass as covariates.

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