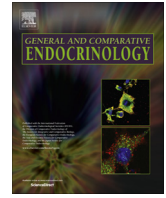




Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Early life stress shapes female reproductive strategy through eggshell pigmentation in Japanese quail



Camille Duval^{a,*}, Cédric Zimmer^a, Ivan Mikšík^b, Phillip Cassey^c, Karen A. Spencer^a

^a School of Psychology and Neuroscience, University of St Andrews, Westburn Lane, St Andrews, Fife KY169JP, United Kingdom

^b Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

^c School of Earth & Environmental Sciences, University of Adelaide, North Terrace SA 5005, Adelaide, Australia

ARTICLE INFO

Article history:

Received 24 May 2014

Revised 15 August 2014

Accepted 17 August 2014

Available online 26 August 2014

Keywords:

Breeding conditions
Developmental history
Early-life stress
Eggshell pigmentation
Japanese quail

ABSTRACT

Physiological constraints on colouration have been widely reported; especially in birds, which trade-off antioxidant responses against colourful costly signals. One female extended phenotypic trait, which might also highlight important physiological trade-offs, is the pigmentation of their eggshells. In ground-nesting species, producing eggs that are visually undetectable by predators is the best camouflage strategy. However, the condition-dependence of eggshell pigmentation, and the pigments role in oxidative stress, may constrain females to trade-off between their antioxidant capacity and maximising the camouflage of their eggs when they deposit eggshell pigments. Developmental stress is one factor that influences female antioxidant capacity, and could lead to variations in eggshell pigmentation that might have crucial consequences on individual fitness if egg crypsis is compromised especially under stressful conditions. We investigated the interaction between developmental and breeding conditions with respect to eggshell pigmentation in Japanese quail. We studied 30 females that bred under both control and stressful conditions, and were exposed to pre- and/or post-natal stress, or neither. Pre- and post-natal stress independently influenced eggshell pigmentation strategies under stressful breeding conditions. Under stressful reproduction, eggshell protoporphyrin concentration and maculation were affected by pre-natal stress, whereas eggshell reflectance and biliverdin concentration were influenced by post-natal stress. These changes may reflect potential adaptive strategies shaped by developmental stress, but additional data on the benefit of egg crypsis in quail, combined with studies on the role of both pigments on chick survival, will help to clarify whether early life stress can enhance fitness through eggshell pigmentation when developmental and reproductive environments match.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Individual variations in physiology, or phenotypic condition, can constrain the development of signals (Hamilton and Zuk, 1982; Grafen, 1990; Von Schantz et al., 1999), leading to trade-offs between physiological functions and the colouration of exaggerated secondary traits. One classical example is the trade-off between antioxidant defence and carotenoid or melanin-based plumage colouration in birds (Von Schantz et al., 1999; Blount et al., 2003; Alonso-Alvarez et al., 2004; Roulin et al., 2011). Colourful traits display seasonal variations long after the completion of signal development and outside the reproductive period (e.g. Korsten et al., 2007; Örnborg et al., 2002). Thus, colourful traits

are labile, and those trade-offs may occur at any point of an individual's lifetime. One female extended phenotypic trait that might be influenced by trade-offs is the pigmentation of a bird's eggshells. In fact, the constraint on colourful eggshell appearance may be stronger than on other colourful phenotypic traits since a female has to allocate pigments during the egg formation, and just prior to laying. This allocation is particularly crucial in species laying on the ground, with interrupted incubation, and which rely on egg appearance to maximise egg crypsis (reviewed in Kilner (2006)).

Several adaptive functions have been suggested for the pigmentation observed in many avian eggshells; including egg crypsis, egg recognition, con-specific signalling (Reynolds et al., 2009), or the enhancement of embryo development and reinforcement of eggshell strength (reviewed in Maurer et al. (2011) and Cherry and Gosler (2010)). The physiological properties of the two main pigments (Gorchein et al., 2009): protoporphyrin, a brown pro-oxidant (Shan et al., 2000) and biliverdin, a blue-green antioxidant

* Corresponding author. Present address: Department of Biological Sciences, Macquarie University, NSW 2109, Australia.

E-mail address: camille.duval07@gmail.com (C. Duval).

(McDonagh, 2001), imply that both pigments could be related to female breeding condition such as food (Duval et al., 2013a), carotenoids (Butler and McGraw, 2013) or calcium availability (Hargitai et al., 2013). For instance, a recent study in the Japanese quail (*Coturnix coturnix japonica*) showed that females under a high quality diet (i.e. under *ad libitum* food compared to food-restricted females) elevated their eggshell deposition of biliverdin, compared to protoporphyrin, while maintaining constant eggshell reflectance (Duval et al., 2013a).

One prominent factor that can influence an individual's antioxidant capacity is the stress experienced during development (Hausmann and Marchetto, 2010; Hausmann et al., 2011; Marasco et al., 2013). Environmental stressors such as an intensified predation risk, or unpredictable changes in resources, are known as acute stressors, and have significant effects on many phenotypic traits that have fitness consequences (reviewed in Killen et al. (2013)). These effects are mediated via the actions of the hypothalamic-pituitary-adrenal axis, which regulates the physiological response to stress, ultimately resulting in the release of glucocorticoids (GCs), such as corticosterone (CORT) in birds. This also promotes several behavioural responses, which serve to enhance survival such as deactivation of territorial behaviour, disintegration of social hierarchies, activation of emergency behaviours (e.g. search of shelter or alternative habitat) and mobilisation of energy reserves (Wingfield et al., 1998; Landys et al., 2006). Sustained elevated GC levels (i.e., chronic stress) can have several deleterious effects on cognitive functions like learning and memory (Bremner, 1999; de Kloet et al., 1999), on individual antioxidant capacity (Marasco et al., 2013), or its neuroendocrine system (e.g. Hypothalamic–pituitary–adrenal axis) (Marasco et al., 2012). Stress can occur at any time, but exposure during critical life-history stages, such as during pre-natal (via maternally transferred GCs) (Sheriff and Love, 2013) and post-natal development (via changes in parental care, nutritional state or sibling interactions) and reproduction, can have long-term consequences (Schoech et al., 2012). Thus, both pre- and post-natal stress may have dramatic negative effects on an individual's fitness (Metcalf and Monaghan, 2001). However, an alternative hypothesis has proposed that maternal stress hormones may shape an offspring's phenotype to program it to better cope in a hostile post-natal environment (Bateson et al., 2004; Gluckman and Hanson, 2004; Monaghan, 2008).

Eggshell pigmentation has been linked to female body condition in some species [e.g. Eastern bluebird (*Sialis sialis*), Blue tit (*Cyanistes caeruleus*), Japanese quail (*C. coturnix japonica*)] (Siefferman et al., 2006; Martínez-de la Puente et al., 2007; Duval et al., 2013a), and it follows that pigmentation may be influenced by both developmental history and the environmental conditions under which a female breeds (Love and Williams, 2008; Cohen et al., 2012). Interestingly, a study on blue tits (*C. caeruleus*) found that females laying more spotted eggs were in lower body condition, had higher cellular concentrations of Heat Shock Proteins (HSP70), which protect cells against oxidative stress, and had marginally lower total immunoglobulin blood levels than those laying less spotted eggs (Martínez-de la Puente et al., 2007). Our previous work in quail (Duval et al., 2013b) showed that female exposure to stress during reproduction induced an increase in eggshell spots brightness (i.e. total reflectance). Thus, female birds may trade-off between their physiological responses and egg appearance (Moreno and Osorno, 2003), and more importantly egg crypsis. In such a ground-laying species, any factor such as stress exposure affecting female development may influence their pigment deposition strategy and potentially impair their ability to maximise egg crypsis, but this idea has never been tested so far.

In the present study, we investigated the effects of early life experiences (both pre and post-natal development) on eggshell

characteristics (i.e. maculation, reflectance and pigment concentrations) and importantly, we assessed how these interact with adult breeding conditions to further influence the eggshell traits. We studied Japanese quail eggs which are covered in brown–red–dish spots and their background colour varies from white to blue–green (Sezer and Tekelioglu, 2009). Both protoporphyrin and biliverdin contribute to eggshell pigmentation (Gorchein, 2012) in the study species, although we assumed that protoporphyrin is primarily responsible for the brown maculation on quail eggshells. In the present study, eggs were laid by females that had been exposed to pre- and/or post-natal stress, or to no such stressors (Zimmer et al., 2013), and that were exposed, or not, to stress during reproduction using an unpredictable food availability paradigm. Very little is known about the nesting biology of Japanese quails in the wild, and females have been observed to use various sites, but preferentially areas with sparse cover. Under semi-captive experimental conditions, Stevens (1961) found that females start nesting in a shallow depression in the ground, and then add straw or weed stems to the nest after each egg is laid. In addition, a recent study experimentally investigating laying substrate choice in quails has demonstrated that females always lay on the substrate that matches the best the colour of eggshell spots and conceals egg outline (Lovell et al., 2013). Thus, both egg pattern (i.e. degree of maculation) and colour (i.e. reflectance) may play a key role in egg crypsis in quail, and any change in egg appearance may compromise the optimality of laying substrate choice in term of egg detectability at the time of laying.

We have previously shown that exposure to a physiological stress during clutch formation only led to an increased eggshell brightness (Duval et al., 2013b) but had no effect on eggshell pigment deposition or maculation. Thus we predicted that females breeding under stress would lay brighter eggshells but maintain their eggshell pigment concentrations and maculation. In addition, precocial birds may be more affected by the environmental stressors experienced *in ovo* as they hatch from the egg fully developed, compared to the stress experienced after hatching (Metcalf and Monaghan, 2001). Therefore, we predicted that pre-natal stress would have a greater influence on eggshell pigmentation than post-natal stress. Finally, if the adaptive view of developmental stress applies for eggshell appearance, then females that experienced pre- or post-natal stress should be better prepared for stressful environments during breeding and should be less affected by stress during reproduction.

2. Materials and methods

All of the procedures conformed to standards established by the local ethics committee at the University of St Andrews (Scotland) and the experiment was conducted under the Animals (Scientific Procedures) Act 1986 (under PIL 70/13261 held by CZ, PIL 70/1364 held by KAS and PPL 60/4068 held by KAS). The eggshells analysed in the present study were obtained from a previous experiment conducted at the University of St Andrews (Zimmer et al., 2013).

2.1. Pre- and post-natal stress

Unrelated fertile Japanese quail eggs ($N = 76$) were injected with either 10 μ l of sterile peanut oil (Control, Ctrl) or with 10 μ l of a sterile solution of CORT (Sigma Aldrich, Poole, UK; concentration CORT: 850 ng/ml) (i.e. pre-natal stress) dissolved in sterile peanut oil at the egg apex, after 5 days of incubation at 37.5 °C and 55% humidity (incubator Ova-Easy 190A, Brinsea Products Ltd., UK). This gave a dose of 8.5 ng of CORT, which increases

endogenous CORT concentrations in the yolk within 1.8 SD above control yolks (see Hayward et al., 2006; Love and Williams, 2008). CORT levels were quantified in a sample of yolks from eggs from the same mothers used in this study ($n = 8$) using radioimmunoassay (RIA) which showed control yolks to contain 8.7 ± 5 (SD) ng/ml (Mullen & Spencer, unpublished data). Experimental injection of 8.5 ng of CORT increased whole yolk CORT levels to 17.1 ± 8.3 (SD) ng/ml (RIA) (Boogert et al., 2013).

Eggs were incubated and the 59 chicks that hatched were randomly allocated to a post-natal food treatment (i.e. post natal stress) at 4 days of age: either food removal for 25% of daylight hours (i.e. 3.5 h) (Cuthill et al., 2000) ($N = 28$) on a random daily schedule for 15 days, or *ad libitum* food ($N = 31$) at all times and group-housed. All of the females ($N = 30$) from these four previous experimental groups (group 1: preCtrl/postCtrl, $N = 6$; group 2: preCtrl/postFood-, $N = 6$; group 3: preCORT/postCtrl, $N = 12$ and group 4: preCORT/postFood-, $N = 6$) were used for the adult food removal treatment (Adult stress). Birds were fed with a standard commercial diet (Layer pellets, ARGO Feeds).

2.2. Adult stress during reproduction

Adult female food removal began between 12 and 16 weeks of age. The 30 females were allocated to one of two further groups (housed in identical rooms): unpredictable food availability (AdFood-) or *ad libitum* food (AdCtrl) (with Ad referring to Adult) and were housed in individual cages ($61.0 \times 44.5 \times 50.8$ cm) for each clutch. Females in the unpredictable group experienced removal of all food items for 25% of daylight hours (3.5 h) on a random schedule between 8 AM and 8 PM over a 30-day period. Controls were provided with *ad libitum* food throughout this breeding period. At the same time, one male was allocated to four females and placed into a cage with a female for 10 min a day in order to obtain fertile eggs. Each female laid two clutches (with an interval of 8 weeks) under the two different conditions (AdCtrl and AdFood-) and the order of each treatment was randomised to control for any effect of the first clutch treatment on the response of the second clutch.

2.3. Egg collection

For both clutches, 3 eggs per female were randomly collected after 10 days of mating, which is the minimum time necessary to obtain fertile eggs (Adkins-Regan, 1995). All 6 eggs (per female) were photographed using a calibrated camera (see further methods). All eggs were then carefully opened along the longitudinal axis using dissecting scissors. The eggshells were collected, washed with distilled water and stored in a dark box to dry at room temperature to avoid direct exposure to light that could cause pigment degradation (Cassey et al., 2011).

2.4. Analysis of eggshell appearance and pigment concentrations

Following a previously published protocol (Duval et al., 2013b), images of the 6 eggs per female were analysed in Matlab (2008a, The MathWorks, Natick). The degree of maculation (spot coverage) present in an egg photograph was estimated as percentage of the foreground (brown spots) and background regions (number of pixels).

Eggshell reflectance was measured between 300 and 700 nm using an Ocean Optics USB4000 Miniature Fibre Optic spectrophotometer (Ocean Optics, Eerbeek), from both eggshell brown spots and background (Duval et al., 2013a,b). Spectra were analysed using a descriptive method (McDonagh, 2001). The spot reflectance spectra of eggshells of Japanese quail exhibit a peak at ~ 630 nm which is consistent with the presence of protoporphyrin IX (Sanz

and García-Navas, 2009). Background spectra show the same peak at 630 nm, and also two more peaks: one around 500 nm, similar to the reflectance spectra of blue-green eggshells (Siefferman et al., 2006), and one in the UV zone at 320 nm. We focused on 3 colour variables: “brightness”, “blue-green chroma” and “red chroma” extracted from these spectral measurements as spectral shape descriptors using Avicol software (Gomez, 2006; Doutrelant et al., 2008; Duval et al., 2013a,b). Brightness was estimated as the total reflectance (R) between the wavelengths 300 and 700 nm, we calculated blue-green chroma (BGC) (Siefferman et al., 2006) as $R_{400-575 \text{ nm}}/R_{300-700 \text{ nm}}$, and red chroma as $R_{595-655 \text{ nm}}/R_{300-700 \text{ nm}}$.

HPLC chromatography (Mikšik et al., 1996) was used to identify and quantify the whole content of eggshell pigments from the 6 eggs collected. Briefly, each eggshell was solubilised (and esterified) and the resulting solution was filtered, 7.5 ml of chloroform and 5 ml of distilled water were added and then the solution was shaken. The different phases were washed until the washing water had neutral pH. The extract was evaporated and reconstituted in 1 ml of chloroform. Standards for the quantification of protoporphyrin (P8293, Sigma, St Louis, MO, USA) and biliverdin (30891, Sigma, St Louis, MO, USA) were treated using the same procedure. Porphyrins were analysed by reversed-phase HPLC using Agilent 1100 LC system (Agilent, Palo Alto, CA, USA). Chromatographic separation was carried out on a Gemini 5u C18 110A column (250×2 mm i.d.; Phenomenex, Torrance, CA, USA). Protoporphyrin was then detected by fluorescence at 405 nm excitation/620 nm emission, whereas biliverdin was detected by absorbance at 375 nm, as it has no fluorescence response. We used LC-MS (i.e. liquid chromatography was directly coupled to mass spectrometry).

2.5. Statistical analyses

We tested the effect of stress during reproduction on female body mass change between the start and the end of each clutch using a Generalised Linear Mixed Model (GLMM). Female body mass change (end-start of the clutch) was included as a dependent variable and adult treatment (AdCtrl or AdFood-) was included as a fixed factor and specified as a repeated factor. Female identity was included as a random factor.

We tested the effect of pre-natal, post-natal and adult stresses on eggshell maculation (e.g. spot coverage), pigment concentration and reflectance using GLMMs. In each model, pre-natal treatment (preCtrl or preCORT), post-natal treatment (postCtrl or postFood-), adult treatment and the interaction between adult treatment and both of the early life treatments were included as fixed factors. Adult treatment was also specified as a repeated factor. Female and egg number were included as random factors.

Finally, a Pearson's correlation was used to test the relationship between biliverdin and protoporphyrin concentrations. GLMMs were fitted using the Mixed PROC in SAS 9.2 (SAS Institute Corporation), after checking for normality and homoscedasticity of residuals. The REML (Residual Maximum Likelihood) was used as the estimation method. Tukey-Kramer multiple comparison adjustment was applied to obtain corrected P -values. An alpha threshold of 0.05 was used and data are presented as means \pm SEMs.

3. Results

3.1. Effect of adult stress on female body mass

When females bred under *ad libitum* food, they gained body mass (12.67 ± 3.83 g, $N = 30$), but when they bred under food

unpredictability, their weight was unchanged (-0.55 ± 4.83 g, $N = 30$) (GLMM: $F_{1,36.8} = 5.65$, $P = 0.02$).

3.2. Effect of pre-natal stress on eggshell traits

There was a significant effect of the interaction between pre-natal and adult stress on eggshell maculation (pre-natal \times adult: $F_{1,117} = 5.10$, $P = 0.03$). Although multiple comparisons did not show significant differences between treatment groups (Table S1), pre-natal control females tended to lay more maculated eggshells under food removal condition, during breeding, than under control conditions. In contrast, pre-natally stressed females maintained eggshell maculation across the two clutches (Fig. 1a, Table S1).

In addition, there was a significant effect of the interaction between pre-natal and adult stress on eggshell protoporphyrin concentration (pre-natal \times adult: $F_{1,81.4} = 4.46$, $P = 0.04$) (Table 1). Pre-natal control females deposited more protoporphyrin under unpredictable feeding conditions than under control conditions ($t_{79} = -3.90$, $P = 0.001$) (Table S1), whereas pre-natally stressed birds did not change pigment deposition across the two treatments ($t_{83} = -0.7$, $P = 0.9$) (Fig. 1b, Table S1). Eggshell biliverdin and pro-

toporphyrin concentrations were positively correlated under both control ($R^2 = 0.77$, $P < 0.001$, $N = 55$) and food-removal ($R^2 = 0.68$, $P < 0.001$, $N = 57$) treatments.

Pre-natal stress did not have any influence on both eggshell spot and background reflectance (all P s > 0.05) (Table 1).

3.3. Effect of post-natal stress on eggshell traits

There was a significant effect of the interaction between post-natal and adult stress on eggshell reflectance (spot red chroma: post-natal \times adult: $F_{1,129} = 5.95$, $P = 0.02$; background red chroma: post-natal \times adult: $F_{1,132} = 5.16$, $P = 0.02$) (Table 1). Although multiple comparisons did not show significant differences between treatment groups (Table S2), post-natally stressed females tended to lay eggs with redder spots (Fig. 2a, Table S2) and redder backgrounds (Fig. 2b, Table S2) under food removal conditions than under control conditions. In contrast, post-natal control females did not change eggshell reflectance when food was unpredictable during reproduction (Table S2) (Fig. 2a and b). Neither brightness nor blue–green chroma were affected by post-natal stress (all P s > 0.05) (Table 1).

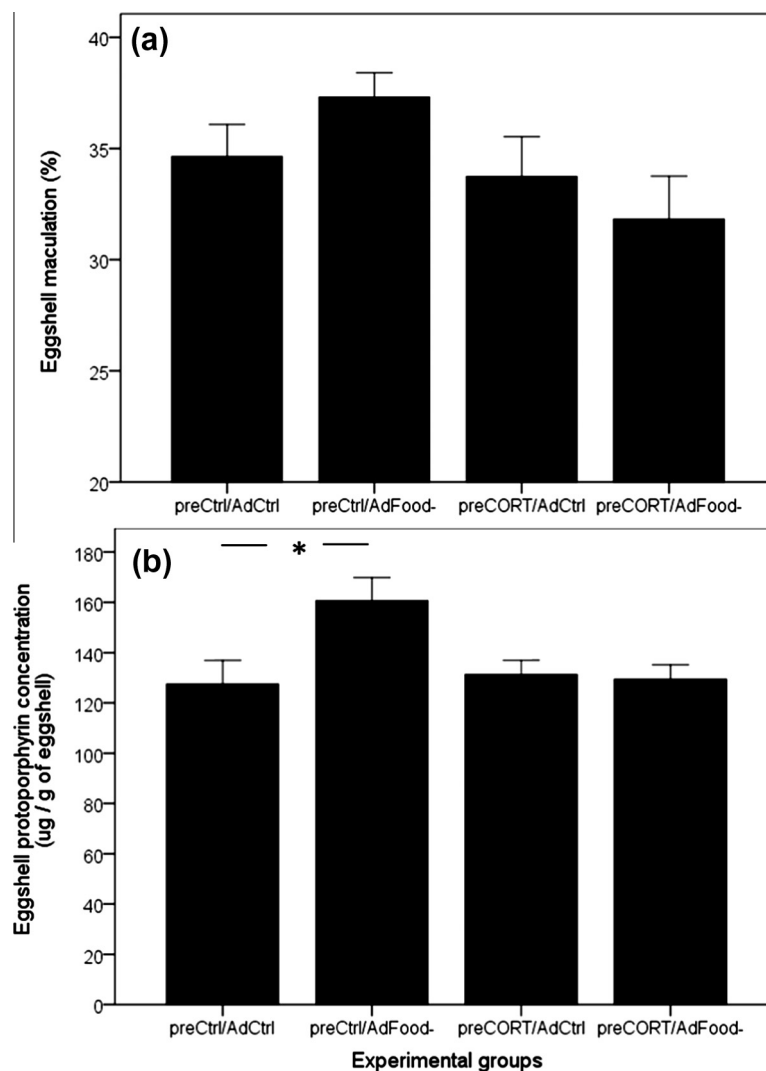


Fig. 1. Effect of pre-natal stress and breeding stress on mean (+1 SE) (a) eggshell maculation and (b) eggshell protoporphyrin concentration, of Japanese quail eggs from four different experimental groups: preCtrl/AdCtrl (pre-natal control, reproduction control), preCtrl/AdFood- (pre-natal control, reproduction stress), preCORT/AdCtrl (pre-natal stress, reproduction control), and preCORT/AdFood- (pre-natal stress, reproduction stress). Asterisks denote statistically significant differences between groups.

Table 1
Effects of pre-natal and post-natal stress and breeding stress on eggshell colouration and pigment concentrations of female Japanese quails (see text for further details). GLMMs were performed to test whether stress influenced eggshell reflectance and pigments concentrations. Bold text indicates statistical significance.

Eggshell trait	Parameter	Factor	dfs	F	P
Spot reflectance	Brightness	Pre-natal stress	1, 26.2	1.98	0.17
		Post-natal stress	1, 26.2	0.12	0.73
		Adult stress	1, 127	1.16	0.28
		Pre-natal × adult stress	1, 127	0.01	0.93
		Post-natal × adult stress	1, 127	3.33	0.07
	Red chroma	Pre-natal stress	1, 25.3	0.11	0.75
		Post-natal stress	1, 25.3	0.00	0.96
		Adult stress	1, 129	0.52	0.47
		Pre-natal × adult stress	1, 129	0.24	0.62
		Post-natal × adult stress	1, 129	5.95	0.02
	Blue-green chroma	Pre-natal stress	1, 26	0.07	0.79
		Post-natal stress	1, 26	0.02	0.89
		Adult stress	1, 126	0.03	0.86
		Pre-natal × adult stress	1, 126	0.07	0.80
		Post-natal × adult stress	1, 126	1.54	0.22
Background reflectance	Brightness	Pre-natal stress	1, 26.1	3.64	0.07
		Post-natal stress	1, 26.1	0.08	0.78
		Adult stress	1, 128	0.11	0.74
		Pre-natal × adult stress	1, 128	0.29	0.59
		Post-natal × adult stress	1, 128	2.21	0.17
	Red chroma	Pre-natal stress	1, 27.7	3.28	0.08
		Post-natal stress	1, 27.7	0.90	0.35
		Adult stress	1, 132	0.03	0.86
		Pre-natal × adult stress	1, 132	0.19	0.66
		Post-natal × adult stress	1, 132	5.16	0.02
	Blue-green chroma	Pre-natal stress	1, 26.3	1.07	0.31
		Post-natal stress	1, 26.3	0.37	0.54
		Adult stress	1, 128	2.17	0.14
		Pre-natal × adult stress	1, 128	<0.01	0.96
		Post-natal × adult stress	1, 128	0.19	0.66
Eggshell pigment	Protoporphyrin	Pre-natal stress	1, 26.4	1.60	0.22
		Post-natal stress	1, 26.4	0.29	0.60
		Adult stress	1, 81.4	9.67	0.003
		Pre-natal × adult stress	1, 81.4	4.46	0.04
		Post-natal × adult stress	1, 81.4	1.75	0.20
	Biliverdin	Pre-natal stress	1, 25.2	0.63	0.43
		Post-natal stress	1, 25.2	0.70	0.41
		Adult stress	1, 47.7	19.55	<0.0001
		Pre-natal × adult stress	1, 47.7	0.31	0.58
		Post-natal × adult stress	1, 47.7	7.38	0.009

Eggshell biliverdin concentration was significantly affected by the interaction between post-natal stress and adult stress (post-natal × adult: $F_{1,47.7} = 7.38$, $P = 0.009$) (Table 1). Post-natally stressed females deposited more biliverdin into their eggshells under food removal conditions than under control conditions ($t_{45} = -4.57$, $P < 0.001$) (Fig. 2c, Table S2). In contrast, post-natal control females did not change their biliverdin allocation strategy when food was removed ($t_{53} = -1.37$, $P = 0.53$) (Table S2) (Fig. 2c).

4. Discussion

Our study revealed that developmental stress interacted with the response to adult reproductive conditions to alter eggshell pigmentation. The degree and direction of eggshell changes differed depending upon the timing of stress. Stress *in ovo* might influence eggshell protoporphyrin deposition and degree of maculation under stress at adulthood, whereas post-natal stress enhanced eggshell biliverdin investment and influenced eggshell reflectance in adult females which bred under stress. Although the adaptive significance of eggshell pigmentation remains to be fully demonstrated in quails, our results provide evidence that early life stress might have long-term effects on some female-related traits that could be crucial in such a ground laying species, such as egg-

shell components and appearance. We note that the small sample sizes in each group may explain why the pairwise comparisons were not statistically significant and the interactions were, thus we cautiously interpret our results in this study.

Japanese quails lay maculated eggs that contain both protoporphyrin and biliverdin (Duval et al., 2013a,b), and may have evolved complex eggshell appearance (i.e. combining colour and patterning) to potentially maximise egg crypsis via disruptive colouration, involving matching nest background colour and visually breaking egg contours (Lovell et al., 2013). However, no study so far has investigated the actual benefit of crypsis strategy for predator avoidance in quail. In addition, no study has yet tested whether a manipulation of egg appearance could influence female nesting choice. Thus, it is possible that changes in eggshell pigmentation strategy could compromise egg crypsis, but additional experimental studies on egg crypsis in quails are needed to support this hypothesis. Here, we found that pre-natal stress interacted with adult breeding stress to influence eggshell maculation and protoporphyrin deposition strategy at adulthood. According to our predictions, females which had experienced pre-natal stress maintained eggshell maculation and concentration of protoporphyrin under stressful breeding condition. In contrast, females from benign pre-natal environments increased eggshell maculation and protoporphyrin deposition under stressful breeding conditions compared to their control clutch. Interestingly, the

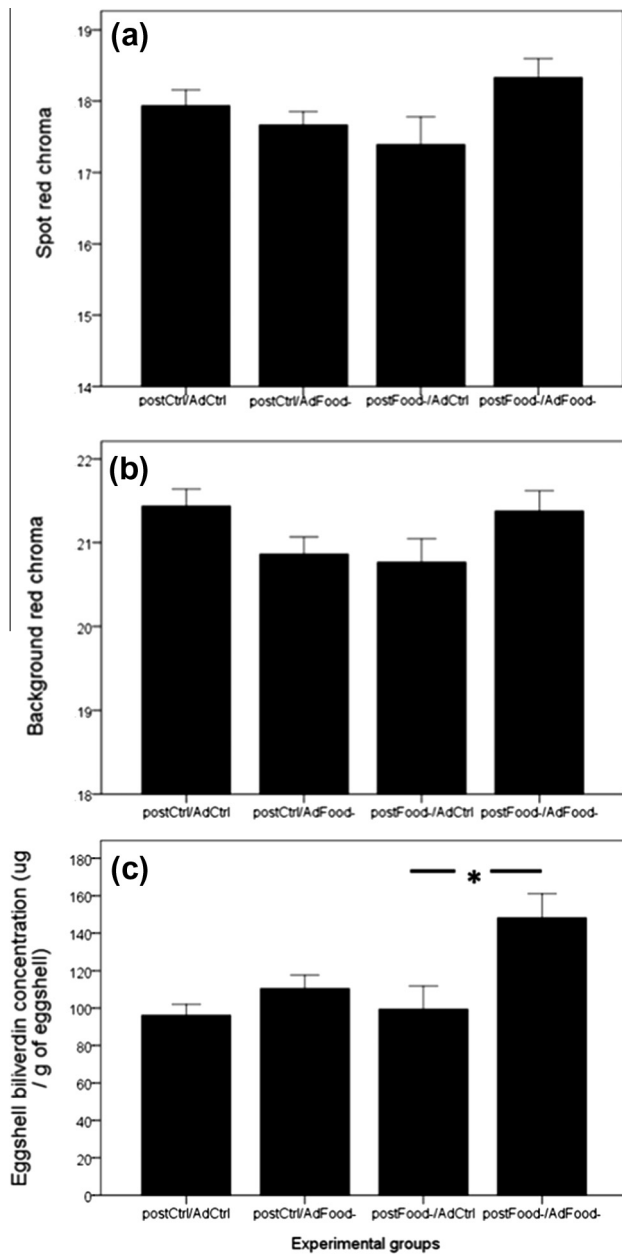


Fig. 2. Effect of post-natal stress and breeding stress on mean (+1 SE) (a) spot red chroma, (b) background red chroma, and (c) eggshell biliverdin concentration, of Japanese quail eggs from four different experimental groups: postCtrl/AdCtrl (post-natal control, reproduction control), postCtrl/AdFood- (post-natal control, reproduction stress), postFood-/AdCtrl (post-natal stress, reproduction control), and postFood-/AdFood- (post-natal stress, reproduction stress) (see text for further details). Asterisks denote statistically significant differences between groups.

removal of food during breeding did not affect female mass, whereas the control condition resulted in females showing a slight body mass increase. As protoporphyrin is a pro-oxidant pigment (Shan et al., 2000), responsible for the brown maculation, it is possible that pre-natal control females may have suffered from an increased oxidative stress following the food removal manipulation during reproduction, and increased the deposition of the brown pigment into the eggshell to eliminate it. Alternatively, both groups could have suffered from an increased oxidative stress and only control birds minimised the cost of it by allocating higher concentrations of protoporphyrin, the pro-oxidant, into the eggshell. Data on female oxidative stress are needed to confirm one of those two hypotheses. Nevertheless, experiencing pre-natal exposure to

stress might have enhanced a female's physiological ability to respond to stress during reproduction, as suggested by the 'environment matching hypothesis' (Monaghan, 2008). In a previously published study (Zimmer et al., 2013), Zimmer and colleagues found that pre-natally stressed individuals exhibited an attenuated stress response, and they proposed that these individuals may be able to respond better to an acute stress during adulthood, such as unpredictable food removal. These females might be able to cope physiologically with an increased allostatic load during reproduction and may also show better antioxidant capacity (Metcalfe and Monaghan, 2001). This could enable individuals to tolerate high concentrations of the pro-oxidant pigment while enduring stress during reproduction, during which they maintain a constant eggshell maculation. The stress experienced by females pre-natally may shape their ability to maintain eggshell appearance constant later in life under stressful conditions. If quails have been shown to know the appearance of their egg and chose laying areas that make their eggs visually less detectable (Lovell et al., 2013), it remains to be demonstrated to what extent this strategy decreases predator attacks and favours individual fitness, and how early life stress could influence the ability of quails to maximise egg crypsis. We found that post-natal stress significantly interacted with adult breeding stress to influence eggshell reflectance and biliverdin deposition. Contrary to our predictions, post-natal stress did not help to maintain a constant eggshell appearance during stressful reproduction. Females that had experienced post-natal stress tended to lay redder eggshells which contained more biliverdin under stressful reproduction. However, eggshell brightness was not affected by female stress exposure. In contrast, post-natal control females kept a constant eggshell reflectance and biliverdin concentration across the two clutch conditions. Biliverdin is a blue-green pigment that possesses antioxidant properties (McDonagh, 2001) and might signal female antioxidant capacity (Moreno and Osorno, 2003). However, the role of biliverdin colouration in brown-spotted eggs remains largely untested. Yet, in Japanese quail, biliverdin may also play a role in brown-spotted eggshells (Duval et al., 2013b) and, for instance, interact with eggshell structure. The slight increase in eggshell red-chroma associated with an increase in the blue-green pigment, biliverdin, was contradictory, but might be explained by a preferential allocation of both pigments in specific layers of the eggshell. Biliverdin may be primarily deposited in the inner layers of the eggshell for eggshell strength and embryo protection, contrary to protoporphyrin which may be preferentially deposited on eggshell surface to shape the maculation. This could explain why eggshell red-chroma, rather than blue-green chroma, slightly increased in eggshells where biliverdin deposition also increased. Nevertheless, the facilitation of eggshell biliverdin deposition in post-natally stressed females could potentially be related to female antioxidant capacity. Recently, it has been shown that post-natal diet restriction diminishes oxidative damage in yellow-legged gull chicks (*Larus michahellis*) (Noguera et al., 2011). It is conceivable that post-natal food removal conferred higher antioxidant capacity to stressed females, which were thus able to allocate higher quantities of biliverdin, the antioxidant pigment, into their eggshells under stress. Besides its hypothetical role as a sexually selected trait (Moreno and Osorno, 2003), biliverdin deposition may confer advantages to the embryo, such as favouring its development, similar to amphibians (Falchuk et al., 2002) and ensuring protection against bacterial infection (Ishikawa et al., 2010), or harmful solar radiation (Lahti, 2008). However, we also found that eggshell reflectance was modified in post-natally stressed females under stressful breeding conditions, which could influence egg detectability by predators that heavily rely on visual cues. Again, we can only speculate to the function of eggshell crypsis via eggshell colour in quails, and more studies are needed to confirm this hypothesis.

5. Conclusion

To conclude, the interaction between development and adult environments is crucial for shaping a phenotypic trait such as eggshell appearance. Both pre- and post-natal stresses have independent effects on female eggshell pigmentation strategy under stress. Protoporphyrin is mainly responsible for eggshell maculation in Japanese quail, and might be essential for egg camouflage (Lovell et al., 2013). Thus, shaping eggshell maculation, which is a major feature of egg crypsis, as early as at the pre-natal stage, could be essential to determine egg and chick survival later in life. In contrast, biliverdin deposition strategy in adults may be more sensitive to post-natal stress, could be costly to females, and might impair other processes such as their ability to keep constant eggshell reflectance while optimising embryo development. We remain cautious regarding our conclusions on egg crypsis in quail as there is no current evidence of the anti-predator benefit of eggshell colour and pattern in the wild. We can only speculate on the effect of early life stress on individual fitness through eggshell pigmentation as a lot more experimental studies are needed to highlight the properties of both biliverdin and protoporphyrin, and in particular the influence of both pigments on the developing embryo. Our study provides the first insight into the importance of female developmental history in shaping their strategy of eggshell pigmentation under stressful breeding environmental conditions, although the fitness benefits of eggshell pigments, maculation and colouration changes have yet to be experimentally demonstrated in quails.

Acknowledgments

We thank the animal technicians from the University of St Andrews for providing care to the birds, and Dr George Lovell for providing the Matlab scripts used for the maculation analysis. Funding was provided by a Birmingham University Teaching Assistantship (to C.D.) and a Biotechnology and Biological Sciences Research Council (BBSRC BBE024459) David Phillips Fellowship (to K.A.S.). P.C. is an Australian Research Council (ARC) Future Fellow (FT0991420).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ygcen.2014.08.013>.

References

- Adkins-Regan, E., 1995. Predictors of fertilization in the Japanese quail, *Coturnix japonica*. *Anim. Behav.* 50 (5), 1405–1415.
- Alonso-Alvarez, C., Bertrand, S., Devevey, G., et al., 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am. Nat.* 164 (5), 651–659.
- Bateson, P., Barker, D., Clutton-Brock, T., et al., 2004. Developmental plasticity and human health. *Nature* 430, 419–421.
- Blount, J.D., Metcalfe, N.B., Birkhead, T.R., et al., 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* 300 (5616), 125–127.
- Boogert, N.J., Zimmer, C., Spencer, K.A., 2013. Pre- and post-natal stress have opposing effects on social information use. *Biol. Lett.* 9 (2).
- Bremner, J.D., 1999. Does stress damage the brain? *Biol. Psychiatry* 45, 797–805.
- Butler, M.W., McGraw, K.J., 2013. Eggshell coloration reflects both yolk characteristics and dietary carotenoid history of female mallards. *Funct. Ecol.* 27 (5), 1176–1185.
- Cassey, P., Hauber, M.E., Maurer, G., et al., 2011. Sources of variation in reflectance spectrophotometry: a quantitative analysis using avian eggshell colour. *Methods Ecol. Evol.* 3, 450–456.
- Cherry, M.I., Gosler, A.G., 2010. Avian eggshell coloration: new perspectives on adaptive explanations. *Biol. J. Linn. Soc.* 100 (4), 753–762.
- Cohen, A.A., Martin, L.B., Wingfield, J.C., et al., 2012. Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends Ecol. Evol.* 27, 428–435.
- Cuthill, I.C., Maddocks, S.A., Weall, C.V., et al., 2000. Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. *Behav. Ecol.* 11, 189–195.
- de Kloet, E.R., Oitzl, M.S., Joëls, M., 1999. Stress and cognition: are corticosteroids good or bad guys? *Trends Neurosci.* 22 (10), 422–426.
- Doutrelant, C., Gregoire, A., Grnac, N., et al., 2008. Female colouration indicates female reproductive capacity in blue tits. *J. Evol. Biol.* 21, 226–233.
- Duval, C., Cassey, P., Mikšik, I., et al., 2013a. Condition-dependent strategies of eggshell pigmentation: an experimental study of Japanese quail (*Coturnix coturnix japonica*). *J. Exp. Biol.* 216 (4), 700–708.
- Duval, C., Cassey, P., Lovell, P.G., et al., 2013b. Eggshell appearance does not signal maternal corticosterone exposure in Japanese quail: an experimental study with brown-spotted eggs. *PLoS One* 8 (12), e80485.
- Falchuk, K.H., Contin, J.M., Dziedzic, T.S., et al., 2002. A role for biliverdin IX alpha in dorsal axis development of *Xenopus laevis* embryos. *Proc. Natl. Acad. Sci. USA* 99, 251–256.
- Gluckman, P.D., Hanson, M.A., 2004. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatr. Res.* 56 (3), 311–317.
- Gomez, D., 2006. AVICOL, a program to analyze spectrometric data. Free executable available at <<http://sites.google.com/site/avicolprogram/>> or from the author at dodogomez@yahoo.fr.
- Gorchein, A., 2012. Quantitative HPLC of pigments of irregularly coloured eggshells: application to aliquots of powdered shell from quail. *Biomed. Chromatogr.* 26 (12), 1605–1607.
- Gorchein, A., Lim, C.K., Cassey, P., 2009. Extraction and analysis of colourful eggshell pigments using HPLC and HPLC/electrospray ionization tandem mass spectrometry. *Biomed. Chromatogr.* 23 (6), 602–606.
- Grafen, A., 1990. Biological signals as handicaps. *J. Theor. Biol.* 144, 517–546.
- Hamilton, W.D., Zuk, L.W., 1982. Heritable true fitness and bright birds: a role for parasites? *Science* 218, 384–387.
- Hargitai, R., Nagy, G., Herényi, M., et al., 2013. Effects of experimental calcium availability, egg parameters and laying order on Great Tit *Parus major* eggshell pigmentation patterns. *Ibis* 155 (3), 561–570.
- Hausmann, M.F., Marchetto, N.M., 2010. Telomeres: linking stress and survival, ecology and evolution. *Curr. Zool.* 56 (6), 714–727.
- Hausmann, M.F., Longenecker, A.S., Marchetto, N.M., et al., 2011. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proc. R. Soc. Lond. B* 279, 1447–1456.
- Hayward, L.S., Richardson, J.B., Grogan, M.N., et al., 2006. Sex differences in the organizational effects of corticosterone in the egg yolk of quail. *Gen. Comp. Endocr.* 146, 144–148.
- Ishikawa, S.I., Suzuki, K., Fukuda, E., et al., 2010. Photodynamic antimicrobial activity of avian eggshell pigments. *FEBS Lett.* 584 (4), 770–774.
- Killen, S.S., Marras, S., Metcalfe, N.B., et al., 2013. Environmental stressors alter relationships between physiology and behaviour. *Trends Ecol. Evol.* 28 (11), 651–658.
- Kilner, R.M., 2006. The evolution of egg colour and patterning in birds. *Biol. Rev.* 81 (3), 383–406.
- Korsten, P., Vedder, O., Szentirmai, I., et al., 2007. Absence of status signalling by structurally based ultraviolet plumage in wintering blue tits (*Cyanistes caeruleus*). *Behav. Ecol. Sociobiol.* 61, 1933–1943.
- Lahti, D.C., 2008. Population differentiation and rapid evolution of egg color in accordance with solar radiation. *Auk* 125 (4), 796–802.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocr.* 148 (2), 132–149.
- Love, O.P., Williams, T.D., 2008. Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Horm. Behav.* 54, 496–505.
- Lovell, P.G., Ruxton, G.D., Langridge, K.V., et al., 2013. Egg-laying substrate selection for optimal camouflage by quail. *Curr. Biol.* 23 (3), 260–264.
- Marasco, V., Robinson, J., Herzyk, P., et al., 2012. Pre- and post-natal stress in context: effects on the stress physiology in a precocial bird. *J. Exp. Biol.* 215 (22), 3955–3964.
- Marasco, V., Spencer, K.A., Robinson, J., 2013. Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance. *Gen. Comp. Endocr.* 191, 239–246.
- Martínez-de la Puente, J., Merino, S., Moreno, J., et al., 2007. Are eggshell spottiness and colour indicators of health and condition in blue tits *Cyanistes caeruleus*? *J. Avian Biol.* 38 (3), 377–384.
- Maurer, G., Portugal, S.J., Cassey, P., 2011. Review: an embryo's eye view of avian eggshell pigmentation. *J. Avian Biol.* 42 (6), 494–504.
- McDonagh, A.F., 2001. Turning green to gold. *Nat. Struct. Mol. Biol.* 8 (3), 198–200.
- Metcalfe, N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* 16 (5), 254–260.
- Mikšik, I., Holáň, V., Deyl, Z., 1996. Avian eggshell pigments and their variability. *Comp. Biochem. Physiol. B* 113 (3), 607–612.
- Monaghan, P., 2008. Early growth conditions, phenotypic development and environmental change. *Philos. Trans. R. Soc. B* 363 (1497), 1635–1645.
- Moreno, J., Osorno, J.L., 2003. Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality? *Ecol. Lett.* 6 (9), 803–806.

- Noguera, J.C., Lores, M., Alonso-Álvarez, C., et al., 2011. Thrifty development: early-life diet restriction reduces oxidative damage during later growth. *Funct. Ecol.* 25 (5), 1144–1153.
- Örnborg, J., Andersson, S., Griffith, S., et al., 2002. Seasonal changes in a ultraviolet structural colour signal in blue tits, *Parus caeruleus*. *Biol. J. Linn. Soc.* 76, 237–245.
- Reynolds, S.J., Martin, G.R., Cassey, P.B., 2009. Is sexual selection blurring the functional significance of eggshell coloration hypotheses? *Anim. Behav.* 78 (1), 209–215.
- Roulin, A., Almasi, B., Meichtry-Stier, K.S., 2011. Eumelanin-and pheomelanin-based colour advertise resistance to oxidative stress in opposite ways. *J. Evol. Biol.* 24 (10), 2241–2247.
- Sanz, J.J., García-Navas, V., 2009. Eggshell pigmentation pattern in relation to breeding performance of blue tits *Cyanistes caeruleus*. *J. Anim. Ecol.* 78 (1), 31–41.
- Schoech, S.J., Rensel, M.A., Wilcoxon, T.E., 2012. Here today, not gone tomorrow: long-term effects of corticosterone. *J. Ornithol.* 153, 217–226.
- Sezer, M., Tekelioglu, O., 2009. Quantification of Japanese Quail eggshell colour by image analysis. *Biol. Res.* 42 (1), 99–105.
- Shan, Y., Pepe, J., Lu, T.H., et al., 2000. Induction of the heme oxygenase-1 gene by metalloporphyrins. *Arch. Biochem. Biophys.* 380 (2), 219–227.
- Sheriff, M.J., Love, O.P., 2013. Determining the adaptive potential of maternal stress. *Ecol. Lett.* 16 (2), 271–280.
- Siefferman, L., Navara, K.J., Hill, G.E., 2006. Egg colouration is correlated with female condition in eastern bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* 59, 651–656.
- Stevens, V.C., 1961. Experimental study of nesting by *Coturnix* quail. *J. Wildl. Manage.* 25 (1), 99–101.
- Von Schantz, T., Bensch, S., Grahn, M., et al., 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* 266, 1–12.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., 1998. Ecological bases of hormone-behavior interactions: the “Emergency Life History Stage”. *Am. Zool.* 38 (1), 191–206.
- Zimmer, C., Boogert, N.J., Spencer, K.A., 2013. Developmental programming: cumulative effects of increased pre-hatching corticosterone levels and post-hatching unpredictable food availability on physiology and behaviour in adulthood. *Horm. Behav.* 64 (3), 494–500.