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ORIGINAL ARTICLE

Patterns of variation in eggshell colour of Mountain Bluebirds (*Sialia currucoides*) provide mixed support for the sexually selected eggshell colour hypothesis

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Abstract Determining the functional significance of ornamental traits and whether they are shaped by sexual selection requires understanding how they vary among individuals, and their relationship with other measures of quality. Conspicuous eggshell coloration is a trait that may have evolved through sexual selection as a signal of female quality. This hypothesis is based on the possibility that the pigment used to colour blue-green eggs is limited and potentially costly to allocate to eggshells. We investigated the signalling potential of blue-green eggshell colour by quantifying patterns of within- and among-clutch colour variation in Mountain Bluebirds (*Sialia currucoides*) to determine if pigment was limited and if egg colour traits are intrinsic to individual females. We also explored relationships over two breeding seasons between eggshell colour and other female traits and investment in eggs. Eggshell colour was highly repeatable within clutches for most measures, and relatively consistent between breeding events in different years, indicating that eggshell colour is an intrinsic trait. Eggs in second clutches within a season had less saturated blue-green colour than in first clutches, and within clutches, later-laid eggs were brighter but generally less blue-green in colour than earlier laid eggs, providing evidence of pigment limitation. However, associations between eggshell colour and other female traits and investment in eggs were less clear, and inconsistent among study years. Our findings suggest that there is potential for blue-green egg colour to function as a signal of female quality, but further study is needed to determine if blue-green eggshell colour is a truly informative signal.

Keywords Eggshell colour · Female traits · Quality signalling · Blue-green

Zusammenfassung

Unterschiedliche Farbmuster der Eier des Berghüttensängers (*Sialia currucoides*) bieten eine unklare Unterstützung der auf geschlechtlicher Selektion beruhenden "Eischalenfarben-Hypothese"

Um die funktionelle Bedeutung schmückender, dekorativer Merkmale zu ermitteln und auch, ob sie ein Ergebnis sexueller Selektion sind, muss man verstehen, wie sie zwischen den einzelnen Individuen variieren und mit anderen qualitativen Merkmalen zusammenhängen. Eine auffällige Eierfärbung ist ein Merkmal, das sich durch sexuelle Selektion als ein Signal für die Qualität des Weibchens herausgebildet haben könnte. Diese Hypothese basiert auf der Annahme, dass das Pigment für blau-grüne Schalenfärbung möglicherweise eine begrenzte Ressource ist und es deshalb aufwendig wäre, diese für die Färbung von Eischalen zu verbrauchen. Für den Berghüttensänger (*Sialia currucoides*) untersuchten wir die mögliche Signalwirkung blau-grüner Schalenfärbungen durch die Quantifizierung der Farbmustervariationen innerhalb eines Geleges einerseits und andererseits zwischen einzelnen Gelegen, um herauszufinden, ob das Pigment begrenzt ist und ob bestimmte Farbmuster für individuelle Weibchen spezifisch sind. Über zwei Brutperioden hinweg untersuchten wir außerdem, ob es Zusammenhänge zwischen den Eierfärbungen und anderen Merkmalen der Weibchen und ihren "Investments" in die Eier gab. Innerhalb

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eines Geleges wiederholte sich das gleiche Farbmuster immer wieder und war ziemlich konsistent für Bruten in unterschiedlichen Jahren, was darauf hinweist, dass die Farbmuster der Eischalen ein intrinsisches Merkmal sind. Eier einer zweiten Brut in der gleichen Brutsaison zeigten eine weniger satte blau-grüne Farbgebung als in den ersten Bruten, und innerhalb eines Geleges waren die später gelegten Eier generell heller und blasser blau-grün als die vorher gelegten Eier, was auf einen begrenzten Pigment-Vorrat hindeutet. Aber mögliche Zusammenhänge zwischen der Schalenfärbung einerseits und anderen Merkmalen der Weibchen oder ihrer „Eier-Investments"waren weniger klar und über die Untersuchungsjahre hinweg widersprüchlich. Unsere Ergebnisse legen nahe, dass es ein Potential für blau-grüne Eifärbung als Signal für eine hohe Güte der Weibchen gibt; aber nur weitere Untersuchungen können letztlich zeigen, ob die blau-grüne Eifärbung wirklich ein Signal mit Informationswert ist.

Introduction

Conspicuous ornamental traits of animals are thought to have evolved through sexual selection (Darwin 1871; Andersson 1994). Such traits are generally costly to produce and maintain (Zahavi 1975), and are not likely to be favoured by natural selection. Instead, ornamental traits evolve because they confer a reproductive advantage through increased access to mates (Andersson 1994). Sexually selected ornaments can advertise competitive ability, hormone levels, and social status, providing valuable information to opponents (Kodric-Brown and Brown 1984). Alternatively, such traits may signal good genes and current condition to potential mates (Andersson 1994). The evolution of ornamental traits has primarily been investigated in males, because male reproductive success often is highly variable and determined chiefly by their ability to gain access to mates, motivating males to compete for such access. Female reproductive success is mainly limited by their ability to produce gametes and raise young, resulting in females being selective when choosing mates (Trivers 1972). However, females of many taxa produce ornamental traits [e.g., nuptial colouration in fish (Baldauf et al. 2011), throat colour in lizards (Weiss 2006), and plumage colour and song in birds (Siefferman and Hill 2005; Karubian 2013)]. Such traits may have evolved through male mate choice, particularly for species where male investment in mating effort (either pre- or post-copulation) is substantial and they cannot invest equally in mating with all available females (Edward and Chapman 2011). While there has been an increase in studies of female traits over the past decade, our understanding of female ornaments is still poor compared to those produced by males, and more research is needed to illuminate the evolution of female ornaments and whether or not they are informative advertisements.

Blue-green eggshell colour is an attribute, produced only by females, that has been proposed as an ornament that advertises female quality and influences male investment in parental care (Moreno and Osorno 2003). Blue-green eggshell colour is the result of biliverdin deposited in the surface layer of eggshells during laying (Sparks 2011). Biliverdin is a metabolically produced antioxidant pigment with the potential to provide important health benefits by scavenging free radicals and preventing damage from oxidative stress (Stocker et al. 1990). As oxidative stress is high during egg laying (von Schantz et al. 1999), female birds may be limited in the amount of biliverdin they can produce and allocate to eggshells. Evidence suggests that eggshell biliverdin is synthesized by the shell gland (Zhao et al. 2006), but little is known about the potential costs of producing it. If biliverdin is limited, and eggshell colour accurately reflects pigment levels as some studies suggest (Moreno et al. 2006; López-Rull et al. 2008; but see Cassey et al. 2012), eggshell colour should be less saturated for eggs laid later within clutches and for second breeding attempts in the same season. There is evidence that blue-green colour of later-laid eggs is lower than that of eggs laid earlier in the laying sequence both in passerine (Moreno et al. 2005; Krist and Grim 2007; López de Hierro and De Neve 2010) and non-passerine birds (Morales et al. 2011), but these findings are not universal (Siefferman et al. 2006; Hargitai et al. 2008; Hanley and Doucet 2009). While studies that directly compare colouration between subsequent breeding attempts within seasons are rare, those that have been conducted have shown a decline in the pigmentation of successive clutches (López de Hierro and De Neve 2010; Honza et al. 2012), providing further evidence of pigment limitation.

For a trait to be a sexually selected signal of intrinsic quality, it should be repeatable within individuals; but if the trait is condition-dependent, it is also likely to be sensitive to resource availability and affected by environmental conditions, and show variation within an individual among years or breeding attempts (Vitousek et al. 2012). Numerous studies have found that egg colour is more consistent within clutches than among clutches (Moreno et al. 2004; Siefferman et al. 2006; Krist and Grim 2007; Soler et al. 2008; Hanley and Doucet 2009; Morales et al. 2011; Honza et al. 2012), but few studies have investigated the repeatability of egg colour between breeding attempts of individual females. Studies of multiple breeding attempts have generally focused on egg recognition as a mechanism for preventing brood parasitism (Honza et al. 2012), or have been conducted using domestic fowl which are not subject to the same array of environmental pressures, and lay eggs continuously rather than in discrete clutches (Dearborn et al. 2012).

If eggshell colour is a meaningful signal of female quality we expect it to co-vary with other female traits associated with quality and investment in reproduction. There is evidence for several of these predictions, but they are far from universally supported. Females that initiate clutches earlier in the season have been found to lay more highly pigmented eggs (Moreno et al. 2006; Honza et al. 2012), providing support for eggshell colour as a quality signal because older, higher-quality females generally breed earlier in the season (Ardia 2005), but other studies have not found such relationships (Siefferman et al. 2006; Hargitai et al. 2008). Measures of structural size and condition also have been found to be positively related to egg colour (Moreno et al. 2005; Siefferman et al. 2006; Krist and Grim 2007: but see Cassey et al. 2008; Hargitai et al. 2008; López-Rull et al. 2008; Johnsen et al. 2011), but few studies have investigated relationships between egg colour and other ornamental traits. The only study to our knowledge that investigated relationships between female plumage and egg colour found that the size of white wing patches of female Collared Flycatchers (*Ficedula albicollis*) were not related to the colour of their eggs (Hargitai et al. 2008). In addition, support for relationships between pre-hatching maternal investment and eggshell colour are mixed. Some studies have found that egg colour was positively related to clutch size (López de Hierro and De Neve 2010), egg mass (Moreno et al. 2006; Siefferman et al. 2006), and maternal investment in yolk components such as antibodies (Morales et al. 2006) and carotenoids (Navarro et al. 2011), but other studies have not found these relationships (Cassey et al. 2008; Hargitai et al. 2008; Krištofík et al. 2013).

We examined the natural patterns of variation in the colour of eggs laid by female Mountain Bluebirds (*Sialia currucoides*) to determine whether there is support in this species for the possibility that blue-green eggshell colour is a sexually selected trait. We quantified eggshell colour and examined variation within and among clutches over two breeding seasons. If pigments were limited, we expected eggshell colour saturation to decline with laying order within clutches, and between subsequent breeding attempts of individual females in the same year. However, if eggshell colour is intrinsic to individual females, we predicted that, although pigmentation may decline, colour variables of clutches laid by the same female should be more repeatable than clutches laid by different females, both between first and second clutches and among years. In addition, we investigated the relationship between eggshell colour and several female traits (clutch initiation date and plumage colour) and measures of their investment in eggs (egg mass and relative yolk volume). If eggshell colour is a signal of female quality and investment, we expected females that initiated clutches earlier in the season and those that had bluer plumage, which has been shown to be a quality signal in this species (Morrison et al. 2014; but see Balenger et al. 2007), would lay clutches of eggs with more saturated blue-green colour. We also predicted that the colour of these clutches would be positively related to the mass and yolk volume of the eggs.

Methods

Study site, species, and general field procedures

We studied Mountain Bluebirds breeding in nest boxes southwest of Williams Lake, BC, Canada (51°N, 122°W), from mid-April to early August in 2011 and 2012. Pairs of nest boxes ($n = 84$ pairs) were spaced ~ 5 m apart to reduce interspecific competition with other cavity nesting species, in particular Tree Swallows (*Tachycineta bicolor*), and pairs of boxes were spaced at least 200 m apart to avoid competition with other bluebirds (Power and Lombardo 1996). The habitat in the study area consisted of arid open grassland with scattered stands of Douglas-fir (*Psuedotsuga menziesii*). Mountain Bluebirds are migratory, medium-sized (~ 30 g), socially monogamous passerine birds with biparental care. At this site, they return from the wintering grounds in early March and begin initiating first clutches in late April. Up to 40% of this population also double broods and lays a second clutch of eggs after successfully raising their first brood (O'Brien and Dawson 2013). Clutch sizes range from two to seven eggs, but clutches of five or six eggs are most common (Power and Lombardo 1996 ; O'Brien and Dawson 2013). Mountain Bluebirds lay eggs ranging in colour from pale blue to nearly turquoise, although white eggs have also been documented (from 2 to 9% of clutches; Peak 2011).

We began monitoring nest boxes early in the breeding season to determine the start of nest construction and subsequent clutch initiation. We weighed each egg using a portable balance (nearest 0.01 g) on the day they were laid, and in 2012, an image of each egg was taken using a digital camera and ovolux (see Ardia et al. 2006). We used ImageJ software to measure the yolk and egg dimensions from the images, and then calculated relative yolk volume by dividing yolk volume ($4/3 \times \pi \times \text{radius}^3$) by the total egg volume (calculated using the equation found in Hoyt 1979). After clutches were complete, nests were not disturbed until day 9–10 of incubation (incubation period is ~ 13 days; Power and Lombardo 1996) when we measured the colour of complete clutches of eggs by quantifying their reflective properties using a spectrometer (details below). Once eggs had hatched, we captured adult females while they were feeding young. For identification, we banded all females with a unique combination of a numbered aluminium band and three coloured plastic bands. Additionally, we collected rump feathers (8–12) and measured their spectral properties (details below).

Colour quantification

We measured the colour of eggs on day 9–10 of the incubation period over the range of wavelengths visible to songbirds (300–700 nm) using an Ocean Optics USB2000 spectrometer (Dunedin, FL, USA) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO, USA). Three measurements of reflectance of each egg, relative to a white standard, were taken in an enclosed dark environment to eliminate ambient light. From these reflectance spectra, we calculated metrics for brightness (total light reflectance from 300 to 700 nm), hue (the wavelength of maximal reflectance), blue-green chroma (relative reflectance from 400 to 575 nm), and ultraviolet (UV) chroma (relative reflectance from 300 to 400 nm) using the package pavo (Maia et al. 2013) in R 3.1.2 (R Core Team 2014), and then averaged the values for each egg. Eggshell blue-green chroma was negatively correlated with UV chroma ($r = -0.82$, $n = 68$, $P < 0.001$) and brightness ($r = -0.77$, $n = 68$, $P < 0.001$), which were positively related ($r = 0.58$, $n = 68$, $P < 0.001$). Eggshell hue was negatively correlated with UV chroma ($r = -0.31$, $n = 68$, $P = 0.01$), but was not related to blue-green chroma ($r = -0.07$, $n = 68$, $P = 0.58$) or brightness ($r = 0.06$, $n = 68$, $P = 0.63$). For among-clutch comparisons, we averaged the colour metrics of all eggs in each clutch. We did not include inviable eggs in clutch averages, as viability influences the quantification of egg colour (Randall and Dawson 2017).

We quantified female feather colour by mounting eight feathers on a non-reflective black background in an overlapping position to mimic their natural positioning on the body, and measuring the spectral characteristics of the feathers from 300 to 700 nm (O'Brien and Dawson 2011). We took three measurements from random locations at the distal tip of the feather sample for each individual, and as with the quantification of eggshell colour, we extracted colour metrics from the raw spectral data using pavo (Maia et al. 2013), and then averaged the values for each metric. For feathers, we calculated brightness, hue, and combined UV-blue chroma (relative reflectance between 300 and 510 nm). These measures were correlated, so we used principal component analysis to collapse these metrics into a single measure of colour. The first principal component (PC1) explained 69.6% of the variation in female feather colour. Brightness loaded positively (0.62) with PC1 as did UV-blue chroma (0.96), while the loading value for hue was negative (− 0.89). Feathers with high PC1 scores, therefore, were bright with high UV-blue chroma and maximum reflectance at shorter wavelengths (bluer).

Statistical analyses

We used linear mixed-effects models to assess if egg colour varied predictably within clutches as a function of position in the laying sequence. We included only complete first clutches that did not contain any undeveloped eggs ($n = 21$ in 2011, $n = 25$ in 2012). To allow data from different clutch sizes to be analysed together ($n = 5$ for 4 egg clutches, $n = 28$ for 5 egg clutches, $n = 13$ for 6 egg clutches) we grouped eggs by their relative laying order (see Krist and Grim 2007). We classified eggs as occurring early (egg 1 in 4- and 5-egg clutches, egg 1–2 in 6-egg clutches), mid (egg 2–3 in 4- and 5-egg clutches, egg 3–4 in 6-egg clutches), or late (egg 4 in 4-egg clutches, egg 4–5 in 5-egg clutches, egg 5–6 in 6-egg clutches) in the laying sequence. Initial models included relative egg position as a repeated measure and clutch identity as the subject to control for the non-independence of eggs within a nest, clutch size as a fixed factor, clutch initiation date as a covariate, and all two-way interactions between egg position and other explanatory variables. Clutch initiation dates (where 1 = 1 January) differed between years (see below), so we centred these dates for each year separately by standardizing to a mean of 0 and a standard deviation of 1 (i.e., Z scores; see Ardia 2005). We used a backwards stepwise approach to remove terms and interactions that did not approach significance ($P > 0.10$), except egg position, which was retained in final models as it was the variable of interest.

We calculated repeatability (Lessells and Boag 1987) of egg colour within clutches ($n = 29$ in 2011, $n = 30$ in 2012) relative to among-clutch variability, and whether average egg colour was consistent within individual females between first and second clutches ($n = 9$) laid in the same year, and first clutches laid in different years ($n = 12$). Finally, we compared the average colour of first and second clutches laid in the same year using a paired t test to determine if brightness, blue-green, and UV chroma differed between first and second clutches of individual females; a Wilcoxon signed-rank test was used to compare eggshell hue values as these data were not normally distributed (Shapiro–Wilks: $W_{10} = 0.81$, $P = 0.02$).

We examined the relationship between the average colour of first clutches ($n = 29$ in 2011, $n = 30$ in 2012) and female traits and investment in eggs using linear mixed-effects models. We included centred clutch initiation date (Ardia 2005; O'Brien and Dawson 2013) and female feather colour as proxies for female age and quality, and egg mass as a measure of investment in eggs. Sample sizes were smaller for models that included feather colour as not all females were captured ($n = 24$ in 2011, $n = 24$ in 2012).

The reproductive patterns for first clutches were different between the 2 years in which egg colour data were collected, likely due to different spring temperature and rainfall patterns (Randall 2016). Across our population in 2011, clutch initiation dates were earlier (3.9 days ± 0.8 SE, $t_{165} = -4.9$, $P < 0.001$) and clutch sizes were larger (0.29 eggs ± 0.11 SE, $t_{152} = 2.62$, $P = 0.01$) than in 2012. Also, the relationship between clutch initiation date and clutch size was different between the 2 years (Spearman's correlations: negatively correlated in 2011, $r_s = -0.31$, $n = 79$, $P < 0.01$; unrelated in 2012, $r_s = -0.09$, $n = 75$, $P = 0.45$). Therefore, we performed analyses of egg colour and reproductive performance separately for each year. We also used linear mixed-effects models to assess the relationship between eggshell colour and relative yolk volume for a subset of clutches in 2012 ($n = 12$). Due to availability of the ovolux device, data on relative yolk volume was collected only for later-laid clutches (clutch initiation date: 17–30 June). We included types of breeding attempts as a fixed factor in analyses of yolk volume, as some clutches were second nests ($n = 7$), some were renests after a failed first clutch ($n = 3$), and others were late first clutches ($n = 2$). For both analyses, we included clutch identity as a random factor and egg number nested in clutch identity as a repeated measure to account for the non-independence and non-random distribution of eggs within nests. We estimated degrees of freedom using the Kenward–Roger method to account for the unbalanced number of repeated measures (Schaalje et al. 2001). Initial models included all covariates, and we used a backwards stepwise approach to remove terms that did not approach significance ($P > 0.10$).

Linear mixed-effects models were performed using SAS 9.3 (SAS Institute Inc.) and all other analyses were completed using SPSS (version 20). We presented means ± 1 standard error, and considered results significant at the $P \leq 0.05$ level.

Results

Eggshell colour generally changed within clutches through the laying sequence as well as between first and second nests (Figs. 1, 2). For within-clutch colour variation, we found that the position of an egg in the laying sequence had a significant effect on the brightness ($F_{2,235} = 6.95$, $P = 0.001$) and blue-green chroma ($F_{2,235} = 3.04$, $P = 0.05$) of the eggshell. Post-hoc comparisons with a Tukey–Kramer correction showed that early laid eggs had lower brightness than mid- ($P = 0.002$) and late-laid eggs ($P = 0.004$; Fig. 1a). Post-hoc comparisons also showed a trend for early sequence eggs to have higher blue-green chroma than mid- ($P = 0.07$; Fig. 1b) and late-laid eggs ($P = 0.07$; Fig. 1b). However, egg position in the laying sequence did not have a significant effect on the UV chroma ($F_{2,235} = 0.37$, $P = 0.70$; Fig. 1d) or hue of eggshells ($F_{2,235} = 0.34$, $P = 0.70$; Fig. 1c). Clutch size and clutch initiation date did not have significant effects on colour differences within clutches (all P values > 0.3) and were removed from final models. In addition to the within-clutch patterns, there was a significant difference between the average colour of first and second clutches for all chromatic colour measures (Fig. 2). Eggs from first clutches had longer wavelength hue values (greener; $P = 0.007$; Fig. 2b), higher blue-green chroma ($t_9 = 3.31$, $P = 0.009$; Fig. 2c), and lower UV chroma ($t_9 = -2.59$, $P = 0.03$; Fig. 2d) than second clutches, but there was no difference in the brightness of eggshells between first and second clutches ($t_9 = -0.41$, $P = 0.69$; Fig. 2a).

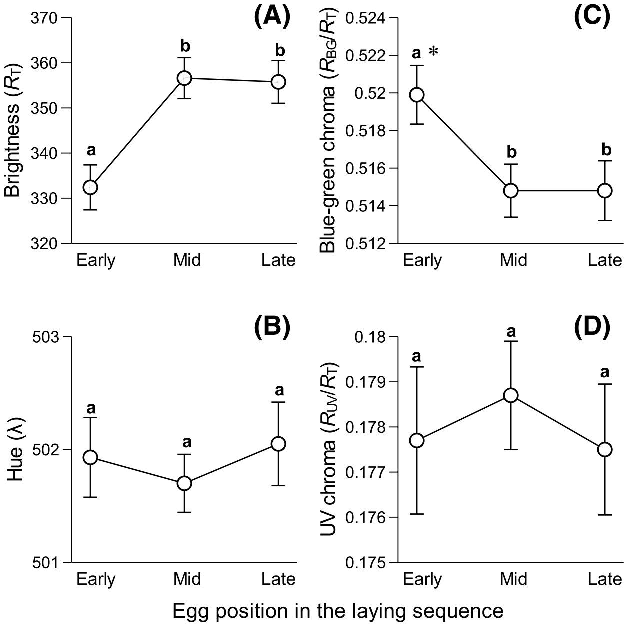

Fig. 1 The mean (± SE) **a** brightness, **b** hue, **c** blue-green chroma, and **d** ultraviolet (UV) chroma of eggshells of Mountain Bluebirds according to relative position in the laying sequence ($n = 238$ eggs from 46 clutches). Early eggs were egg 1 for four- and five-egg clutches, and egg 1–2 for six-egg clutches. Mid-sequence eggs were egg 2–3 in four- and five-egg clutches, and 3–4 in six-egg clutches. Late eggs were egg 4 in four-egg clutches, egg 5 in five-egg clutches, and egg 5–6 in six-egg clutches. Significant differences ($P < 0.05$) determined by post hoc comparisons are indicated above the error bars by different letters (an asterisk indicates a near-significant trend, $P < 0.10$). See "Methods" for details of the calculation of eggshell colour variables

Blue-green chroma and brightness of eggshells were consistent within clutches and between first nests of individual females in different years, but were not repeatable between first and second clutches in the same year (Table 1). UV chroma and hue were also consistent within clutches, but were not repeatable between clutches laid by the same female within a season or in two different years (Table 1).

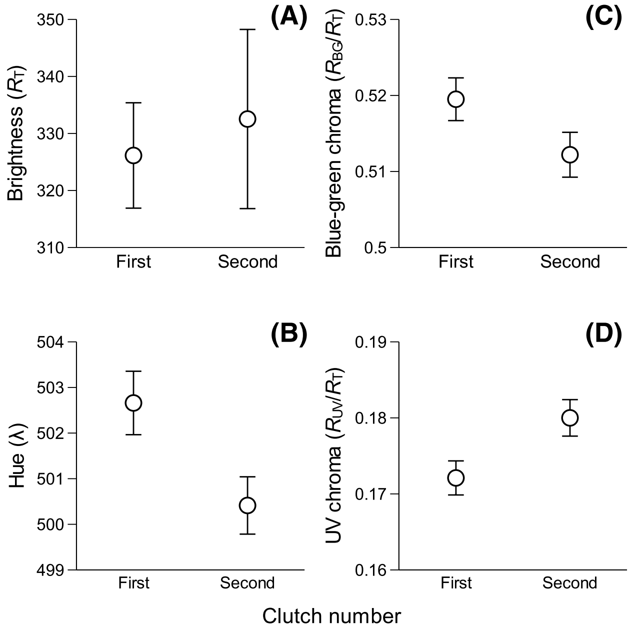

Fig. 2 Mean (± SE) **a** brightness, **b** hue, **c** blue-green chroma, **d** and ultraviolet (UV) chroma of eggs within clutches of Mountain Bluebirds in first and second breeding attempts within the same season (n = 10 females). See "Methods" for details of the calculation of eggshell colour variables

Overall, within-clutch colour was more consistent than among-clutch colour, and eggshell colour was repeatable for most measures of colour for first clutches between years, but did not show high similarity between first and second clutches of individual females in the same year.

Natural variation in eggshell colour was not consistently related to timing of breeding, investment in eggs, or female traits. In 2012, clutch brightness increased with later clutch initiation dates ($F_{1,28.1} = 6.13$, $P = 0.02$; Fig. 3a), and in 2011 there was a trend for females with brighter, more UV-blue feathers (large, positive PC1) to lay clutches of eggs with higher blue-green chroma ($F_{1,21.9} = 3.96$, $P = 0.06$; Fig. 4c), but neither clutch initiation date nor female feather colour were related to any other colour variable (all P values > 0.19; see Figs. 3, 4). Additionally, egg mass was negatively related to eggshell brightness in both years (2011: $F_{1,140} = 4.27$, $P = 0.04$; 2012: $F_{1,142} = 4.06$, $P = 0.05$) and there was a trend in 2012 for heavier eggs to have lower UV chroma ($F_{1,139} = 3.27$, $P = 0.07$), but egg mass was not related to UV chroma in 2011, or to blue-green chroma and hue in either year (all P values > 0.28). Yolk volume was related to eggshell brightness ($F_{1,44.5} = 6.44$, $P = 0.01$), UV ($F_{1,42.5} = 6.43$, $P = 0.02$), and blue-green chroma ($F_{1,43.7} = 9.77$, $P = 0.004$), but not eggshell hue ($F_{1,44.9} = 0.61$, $P = 0.44$). Eggs with shells that were less bright and had higher blue-green chroma and lower UV chroma had higher relative yolk volumes (Fig. 5). Relative yolk volume was significantly related to clutch initiation date in all models (all P values < 0.04), decreasing with later clutch initiation dates, but the nest attempt type (first, second, renest) did not affect relative yolk volume in any model (all P values > 0.80).

Discussion

The patterns of variation we observed within clutches, and between first and second clutches within a season, suggest that biliverdin, the pigment used to colour blue-green eggshells, may be limited in Mountain Bluebirds. Within clutches, brightness increased in a non-linear fashion through the laying sequence, while there was a trend for blue-green chroma to decrease in mid- and late-laid eggs, indicating a reduction in the pigmentation of later laid eggs if eggshell colour accurately reflects the quantity of pigments deposited in eggshells (Moreno et al. 2006; López-Rull

Table 1 Results of repeatability analyses (Lessells and Boag 1987) of eggshell colour within clutches, average colour of first and second clutches in the same year, and first breeding attempts between years (2011 and 2012) for individual female Mountain Bluebirds

Comparison	Variable	Repeatability	*F*	*df*	*P*
Within clutches (*n* = 59)	Brightness	0.68	12.6	58, 238	< 0.001
	Blue-green chroma	0.81	23.8	58, 238	< 0.001
	UV chroma	0.33	3.66	58, 238	< 0.001
	Hue	0.51	6.61	58, 238	< 0.001
Between first and second clutches (*n* = 10)	Brightness	0.29	1.83	9, 10	0.18
	Blue-green chroma	0.47	1.57	9, 10	0.07
	UV chroma	− 0.10	0.82	9, 10	0.62
	Hue	0.10	1.23	9, 10	0.37
Between years (*n* = 14)	Brightness	0.81	9.45	11, 12	< 0.001
	Blue-green chroma	0.69	5.51	11, 12	0.003
	UV chroma	0.32	1.92	11, 12	0.14
	Hue	0.08	1.17	11, 12	0.39

See "Methods" for details of the calculation of colour variables and repeatability analyses

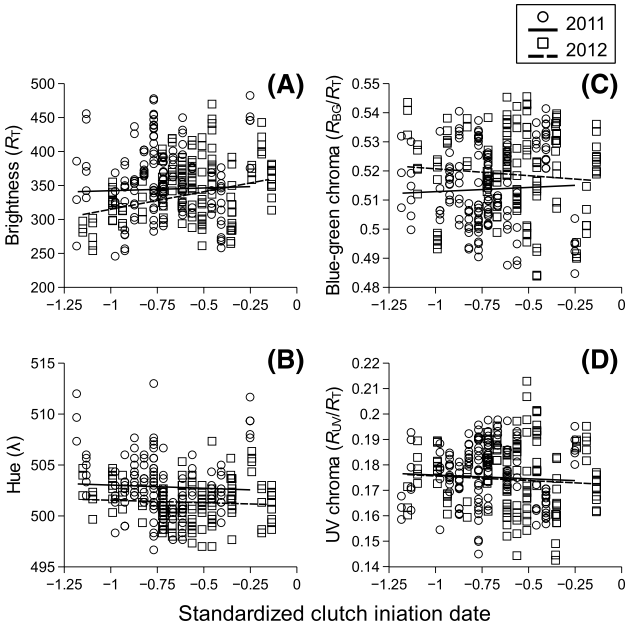

Fig. 3 Relationship between clutch initiation date and eggshell **a** brightness, **b** hue, **c** blue-green chroma, and **d** ultraviolet (UV) chroma of Mountain Bluebirds in 2011 (n = 151 eggs from 29 clutches) and 2012 (n = 146 eggs from 30 clutches). See "Methods" for details of the calculation of eggshell colour variables and standardize initiation date

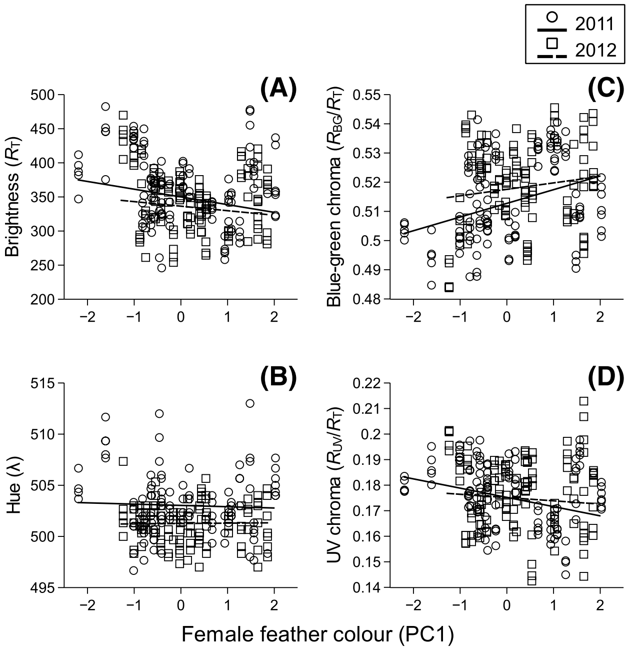

Fig. 4 Relationship between female feather colour (PC1) and eggshell **a** brightness, **b** hue, **c** blue-green chroma, and **d** ultraviolet (UV) chroma of Mountain Bluebirds in 2011 (n = 122 eggs from 24 clutches) and 2012 (n = 117 eggs from 24 clutches). See "Methods" for details of the calculation of colour variables for eggshells and female feathers

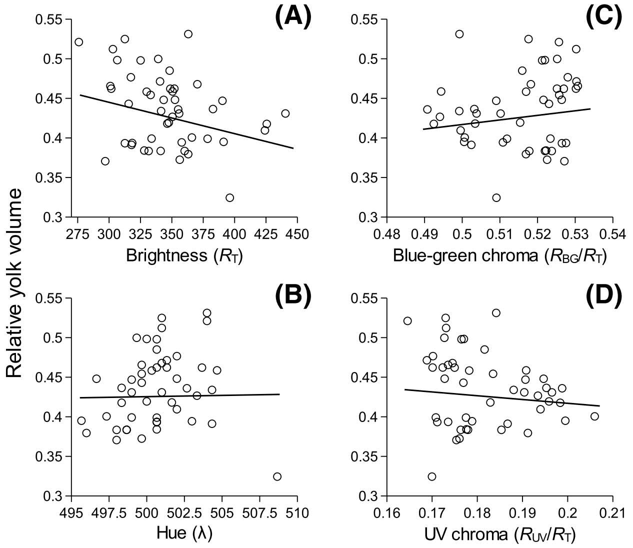

Fig. 5 Relationship between relative yolk volume and eggshell **a** brightness, **b** hue, **c** blue-green chroma, and **d** ultraviolet (UV) chroma of Mountain Bluebirds measured in 2012 (n = 48 eggs from 12 clutches). See "Methods" for details of the calculation of eggshell colour variables and relative yolk volume

et al. 2008: but see Cassey et al. 2012). A similar non-linear decrease in the blue-green chroma of eggshells was found in both Pied (*Ficedula hypoleuca*; Moreno et al. 2005) and Collared Flycatchers (Krist and Grim 2007). A decrease in pigmentation was also found between the first and second eggs of blue-footed boobies (*Sula nebouxii*; Morales et al. 2011), a seabird which generally lays only two eggs. However, three other studies that have found an increase in the pigmentation of later laid eggs in Eastern Bluebirds (*Sialia sialis*; Siefferman et al. 2006), Collared Flycatchers (Hargitai et al. 2008), and Ring-billed Gulls (*Larus delawarensis*; Hanley and Doucet 2009). Furthermore, we did not find a clear pattern for all colour metrics; there were no differences in the hue or UV chroma of eggshells across the laying sequence.

The results of comparisons between first and second nests also indicated that eggshell pigments may be limited. Average clutch blue-green chroma was significantly lower in second nests compared to first nests, UV chroma was higher, and average hue values were shorter (blue-shifted). However, average brightness did not change between first and second clutches. The lower levels of pigments could be the result of pigment depletion from having laid a first clutch of eggs, stress from having incubated and fed nestlings, changing environmental conditions later in the breeding season, or a combination of these factors. Replacement clutches of Great Reed Warblers (*Acrocephalus arundinaceus*) contained brighter eggs (lower pigmentation) than first clutches (Honza et al. 2012), suggesting that laying more eggs alone without the stress of brood rearing is enough to cause pigmentation to decline. Another possibility is that the later initiation dates associated with replacement clutches and second nests result in lower levels of investment due to the declining reproductive value of the offspring (Drent and Daan 1980) or lower food availability as there is evidence that eggshell colour is sensitive to food availability during laying (Randall 2016).

Eggshell colour, particularly blue-green chroma, was consistent both within clutches and between first clutches of the same female laid in different years. The repeatability of eggshell coloration indicates that there may be a genetic basis for eggshell coloration, as found in previous studies (Collias 1993; Morales et al. 2010), and is also suggested by observations that female Mountain Bluebirds that lay white eggs will do so consistently throughout their lifetime (O'Brien and Dawson, unpublished data). However, we found that repeatability of eggshell colour was higher (for all metrics) within clutches than between breeding attempts of the same female, with eggshell colour being particularly inconsistent between first and second clutches in the same breeding season. These findings, along with results showing that colour is more consistent within than among clutches (Moreno et al. 2004; Siefferman et al. 2006; Krist and Grim 2007; Soler et al. 2008; Hanley and Doucet 2009; Morales et al. 2010; Honza et al. 2012), suggests that variation among clutches is influenced both by female identity (e.g., Dearborn et al. 2012) and conditions during a given breeding attempt, such as food availability and individual condition (Honza et al. 2012).

We found that eggshell colour was related to female traits, but not consistently. In 2012, clutch brightness increased with later clutch initiation dates, suggesting a decrease in eggshell pigmentation with later clutch initiation dates, as would be predicted if eggshell colour is a signal of female quality. A similar seasonal pattern was found in the eggs of Great Reed Warblers which also laid brighter eggs later in the season (Honza et al. 2012), but other studies have shown either no relationship between egg colour and clutch initiation date (Siefferman et al. 2006) or an increase in blue-green chroma later in the season (Hargitai et al. 2008). We also found that in 2011, female birds with bluer rump feathers laid eggs with higher blue-green chroma. These results are intriguing because there is evidence that structural plumage is condition-dependent (McGraw et al. 2002) and there is some evidence that female plumage colour may be sexually selected in Mountain Bluebirds (Morrison et al. 2014; but see Balenger et al. 2007), and a closely related species, Eastern Bluebirds (Siefferman and Hill 2005). A relationship between feather colour and egg colour could represent a meaningful and complex multicomponent signal, as the traits are produced at different stages of the annual cycle.

The relationship between measures of eggshell colour and egg mass, which is often used as a measure of investment in eggs, particularly in birds (e.g., Wiebe and Bortolotti 1995), and eggshell colour and relative yolk volume, which is also an established measure of egg quality (Ardia et al. 2006), provide support for eggshell colour as a meaningful signal. In both study years, eggs with brighter shells weighed less. Egg mass was not significantly related to other measures of eggshell colour in either year, but there was a trend in 2012 for eggs with higher UV chroma to weigh less. Additionally, relative yolk volume was strongly and positively related to eggshell blue-green chroma and negatively related to brightness and UV chroma, as would be predicted if eggshell colour saturation advertised female investment in eggs. Moreno et al. (2006) found that food supplementation resulted in eggs that were both heavier and had higher blue-green chroma, but two other studies of passerines found that egg mass correlated very weakly (Cassey et al. 2008), or not at all, with eggshell colour (Hargitai et al. 2008). More convincing relationships have been found between the colour of eggshells and the composition of eggs such as concentrations of antibodies (Morales et al. 2006) and carotenoids of yolks (Navarro et al. 2011; but see Cassey et al. 2008), suggesting that egg colour may be more related to maternal investment in yolk than overall egg mass and egg size, which is often highly consistent within individuals (Christians 2002).

The patterns of natural egg colour variation in Mountain Bluebirds suggest that blue-green egg colour has the potential to function as a signal of female quality. The within-clutch decline in pigment-associated colour through the laying sequence and the change in colour between first and second nests imply that the pigments responsible for blue-green eggshell coloration are limited. In addition, the repeatability of eggshell colour within and between clutches of individual females suggests that eggshell colour has an intrinsic aspect, but is influenced by current conditions. However, the information content of egg colour is still unclear. Females that initiated clutches earlier and had bluer rump feathers laid eggs with more saturated colour, but these findings were not consistent between seasons or among colour metrics. Nonetheless, eggshell colour does seem to reflect investment in eggs. Egg mass was negatively related to eggshell brightness, and relative yolk volume was positively related to eggshell blue-green saturation. Further work is needed to determine if egg colour is merely a by-product of physiological processes or an informative signal shaped by sexual selection.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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