AVIAN BIOLOGY RESEARCH **6** (2), 2013 163–177

Eggshell conspicuousness is related to paternal brood patch vascularisation in the American thrashers

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ABSTRACT

Conspicuous blue-green egg colouration has been of interest to scientists and naturalists for more than a hundred years; however, the function of this trait has never been fully understood and has spurred extensive debate. The American thrashers exhibit great variation in egg colouration and patterning, and also show variation in male brood patch development. A recent hypothesis suggests that conspicuous egg colouration has evolved as the product of sexual conflict, where females may create conspicuous eggs to obtain greater amounts of male care, either in the form of male incubation or incubation assistance to prevent their eggs from being vulnerable to visually orienting predators and brood parasites. The American thrashers, a genus comprising of 10 species, exhibits striking variation in egg colouration and patterning, and also show variation in male brood patch development. By using avian visual modelling, I show that blue-green chroma of American thrasher eggs is positively related to their degree of conspicuousness against a nest background, while brown chroma is negatively related. Interestingly, the degree of male brood patch vascularisation is directly related to eggshell conspicuousness in American thrashers. I suggest that in the American thrashers, male brood patch vascularisation has evolved in response to conspicuous eggshell colouration to mitigate visual risks. Here I show that the degree of male brood patch vascularisation is directly related to relative paternal incubation effort in more than 300 North American breeding birds. This suggests that American thrasher species that possess both conspicuous eggs and brood patches, have most likely evolved to keep these conspicuous nest contents concealed, thereby reducing risk of visual detection. I suggest that these patterns are not unique to thrashers, but may extend to several avian families.

Keywords: blackmail hypothesis, brood patch, crypsis, egg colour, nest reflectance, paternal investment

1. INTRODUCTION

Conspicuous advertisements may be practical when seeking a mate, but they are certainly less appropriate for earlier life stages, particularly vulnerable birds' eggs (Wallace, 1871). Nonetheless, many bird eggs appear conspicuous, which drew the attention of early biologists and naturalists (McAldowie, 1886; Sorby, 1875; Wallace, 1889), because conspicuous colours may alert predators (Haskell, 1996; Lack, 1958; Verbeek, 1990). Numerous single-species studies suggest that eggshell colouration poses a predation cost (Blanco and Bertellotti, 2002; Castilla *et al.*, 2007; Magige *et al.*, 2008; Mayer *et al.*, 2009; Nguyen *et al.*, 2007; Sánchez *et al.*, 2004; Solís and de Lope, 1995; Yahner and Mahan, 1996), and egg colouration is an important predictor of frequency of brood parasitism and host suitability within European passerines (Avilés *et al.*, 2006). In addition to these costs imposed by predation and brood parasitism, the blue-green eggshell pigment, biliverdin (Gorchein *et al.*, 2012; Sorby, 1875; Zhao *et al.*, 2006) may also come at a physiological cost (Hanley *et al.*, 2008; Miksik *et al.*, 1994; Miksik *et al.*, 1996; Morales *et al.*, 2008). Biliverdin has antioxidant-like properties *in vitro* (Kaur *et al.*, 2003) and the antioxidant capacity of the laying female is related to the colour of her eggs (Hanley *et al.*, 2008; Morales *et al.*, 2008). Since avian nest predation and parasitism significantly impact nesting success in many passerine species (Davies, 2000; Schmidt, 1999) and females pay high oxidative costs during the laying period, we should expect selection against these conspicuous blue-green eggs. Given these selection pressures against blue-green colours, why are blue-green eggs so common, particularly in open-nesting species (Kilner, 2006)?

Many hypotheses have been proposed to answer this question. The role of conspicuous egg colouration may be explained by aposematism (Cott, 1948; Swynnerton, 1916), crypsis (Lack, 1958; Tinbergen, 1962; Wallace, 1889), thermoregulation (Bakken *et al.*, 1978; McAldowie, 1886) or egg recognition (Birkhead, 1978; Krist and Grim, 2007; Øien *et al.*, 1995; Soler and Møller, 1996); however, many of these hypotheses lack experimental support or have been discredited (Götmark, 1992; Lack, 1958; Underwood and Sealy, 2002; Weidinger, 2001). A more recent hypothesis, termed the sexual signalling hypothesis, suggests that blue-green eggshell pigmentation serves

doi: 10.3184/175815513X665926

to signal female quality because only the highest quality females can afford to deposit biliverdin, a potentially important antioxidant, during the oxidatively stressful laying period (Moreno and Osorno, 2003). In contrast to most previously proposed hypotheses, this hypothesis has been supported in a variety of birds (recently reviewed in Cherry and Gosler, 2010); however, this support is mixed (Reynolds *et al.*, 2009).

Another recently proposed, yet untested, hypothesis suggests that these conspicuous eggshell colours evolved through sexual conflict (Hanley *et al.*, 2010). This hypothesis has been termed the blackmail hypothesis, and suggests that conflict load placed on the mated pair through the creation of conspicuous eggs should elicit a response from the male to help keep conspicuous nest contents concealed. The hypothesis predicts that males may either share incubation duties or feed their mates on the nest in response to the risk of nest detection. A critical prediction of this hypothesis is that males respond to intraspecific variation in eggshell conspicuousness by covering the eggs during the nesting period, through incubation or incubation assistance. Male response to perceived risks at the intraspecific level should result in interspecific differences in both egg traits and male behaviour. Support for this prediction may also be taken as support for the sexual signalling hypothesis, and therefore will not distinguish the sexual signalling hypothesis from the blackmail hypothesis. However, support of this prediction will add to our knowledge of the former and is a necessity for the latter; therefore, in this paper I use a theoretical approach to examine this prediction in American thrashers. Unfortunately, there is still a great deal that we do not understand about the role males play during the incubation period, which makes some of these tests particularly challenging. However, we do know that incubation is costly (Coleman and Whittall, 1988; Hanssen, *et al.*, 2005). A recent comparative analysis showed that egg temperature generally does not reach that of brood patch temperature (Deeming, 2008), also suggesting a high cost of incubation. In addition, we know that the onset of brood patch development is hormonally controlled (Jones, 1971), and the mechanism differs among species where only the female incubates, where the male and female incubate, and where the male incubates alone (Selander, 1963). This hormonal basis for incubation behaviour establishes a mechanism by which egg conspicuousness and egg covering behaviours may covary over evolutionary time. However, there is great variation in paternal care in birds (Cockburn, 2006), and relatively little is known about the relationship between male brood patches and their incubation behaviour.

For example, in 1957Alexander Skutch reviewed incubation behaviour in birds and argued against a "correlation between incubation and the presence of a brood-patch in the males" (Skutch, 1957); however, there is currently little evidence to support or refute this suggestion. While male incubation confers benefits to offspring (Reid *et al.*, 2002; Verner and Willson, 1969), there are thermodynamic and metabolic costs associated with incubation and brood patch development (de Heij *et al.*, 2006; Turner, 1991), and male participation in incubation reduces the amount of time they can spend on other activities (Verner and Willson, 1969). This is most likely why the incubating sex generally develops a brood patch (Jones, 1971), suggesting that males incubate their eggs in species where males develop brood patches. Therefore, it is not surprising in light of these physiological, developmental, and opportunity costs associated with incubation that brood patches do not develop in either male or female brown-headed cowbirds (*Molothrus ater*) (Pyle, 1997), an obligate brood parasite where neither sex incubates their eggs. I predict that the degree of vascularisation of male brood patches should be directly proportional to relative male incubation effort. If this prediction is supported, I will use brood patch development as a measure of paternal investment.

The blackmail hypothesis suggests that egg detection by predators or brood parasites and paternal investment both exert greater selection on the most conspicuous eggs in the population; however, these extreme values are generally ignored and emphasis given to mean values in most statistical analyses (Figure 1). How drastically this may influence our interpretation of egg colour and comparative research in general remains relatively understudied. In this evolutionary scenario, when males are presented with conspicuous nest contents, they may either not participate in keeping nest contents covered, or help to keep their eggs incubated and hidden from view. If the males do not participate, directional selection from visually orienting predators will select for increasingly cryptic eggshells (Figure 1A). If males do provide assistance to conspicuous nest contents, those eggs will have greater incubation constancy leading to higher success, through reduced predation and more consistent incubation temperatures (Hanley *et al.*, 2010; Reid *et al.*, 2002), providing directional selection for increasingly conspicuous eggs (Figure 1B). Most comparative analyses focus on trait means, which is generally appropriate if the species, or populations, have different trait means but similar variances (Figure 1C). However, there is growing support for considering both between and within trait variation in comparative analyses (Garamszegi and Møller, 2010; Hadfield and Nakagawa, 2010). Since the blackmail hypothesis proposes that both selection pressures operate directionally on the same tail of the population, species may have the same mean but our expectation on how they would respond to selection could differ drastically if their variances are not equivalent (Figure 1D). Although many comparative techniques are robust against the assumption of heterogeneity of variance (Zuur *et al.*, 2010; Pinheiro and Bates, 2010), it seems prudent to investigate selection pressures across the population since there should be a gradient of selection pressure.

To examine how this focus on mean trait values may impact conclusions from traditional comparative

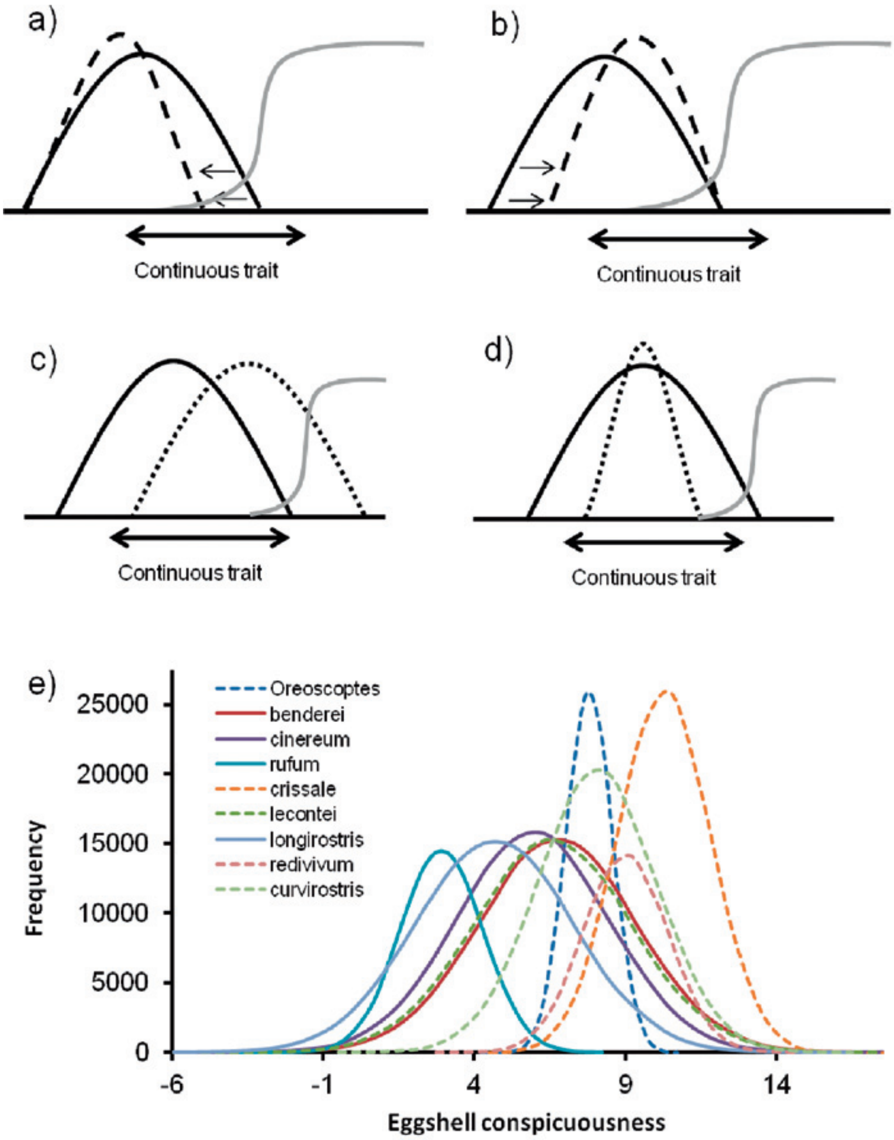

Figure 1 When comparing traits between organisms we often focus on trait means; however, the selection often operates on extreme values. (a) If conspicuous nest contents (solid black line) are not protected effectively by their parents, we expect that selection pressure from visually orienting nest predators (grey histogram) would provide directional selection for enhanced crypsis (dotted line). (b) However, parents may provide greater nest attendance for conspicuous eggs. This would lead to developmental benefits for their offspring and a reduction in the visual risk associated with conspicuous eggshells, resulting in directional selection in the opposite direction. (c) When two populations (*i.e.*, species, or diverging subspecies) have different mean trait values but identical variances, comparing the mean trait values should usually tell us something meaningful about how these species respond to selection, for example from a visually orienting predator (grey line). (d) However, there are cases where two populations have identical means (dotted *versus* solid black lines) but different variances, and in these cases our expectation with respect to how they would respond to visually orienting predators (grey line) differ, and examining differences in the mean would be inadequate. (e) Histograms of eggshell conspicuousness for species measured in this study to illustrate differences in means and variances of eggshell conspicuousness. These represent populations ($N = 100{,}000$) that were generated randomly in R (R Development Core Team, 2010), using the mean and standard deviation in eggshell conspicuousness (calculated across a range of potential nests) for each species. There are multiple cases where examining the mean alone would be inadequate in understanding selection pressures operating on these eggs. Those eggs that are most blue-green are represented by dashed lines, while brown and white eggs are represented by solid lines. Species laying blue-green eggshells tended to have a greater overall conspicuousness score, but also reduced variance, a pattern that may be expected from directional selection for greater conspicuousness.

analyses, I generated a range of eggshell conspicuousness values for each species. If selection is operating on the most conspicuous tail of the population across species (Figures 1A and B), we would expect that our evolutionary models should be strongest when focused on these values, rather than the mean trait values. I generated this conspicuousness range by adjusting potential nest backgrounds from very dark (low overall light intensity) to very light nests (relatively high light intensity). Then comparative analyses were applied across this range of eggshell conspicuousness. Here I use the American thrashers, which exhibit dramatic variation in the

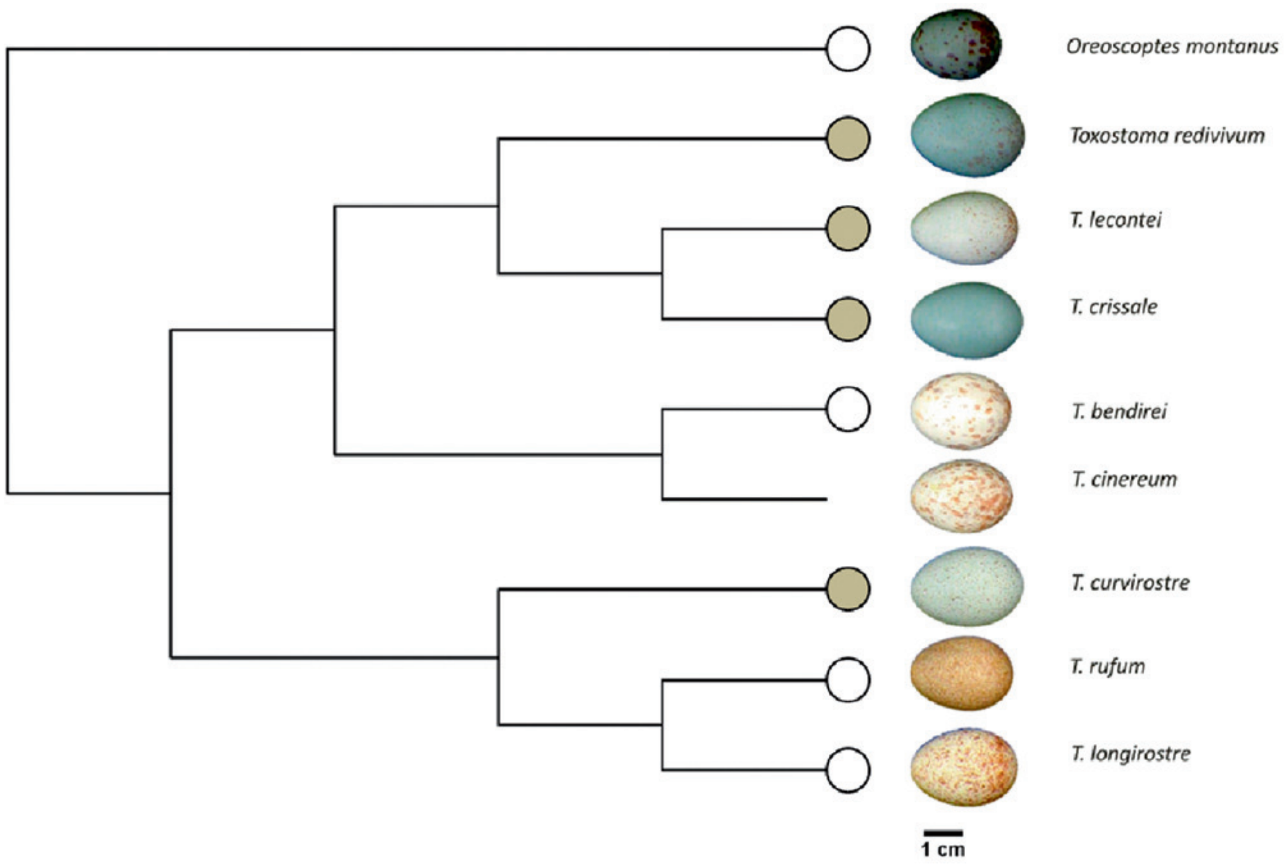

Figure 2 The American thrashers (*Toxostoma* spp.) exhibit a broad diversity of egg colouration and patterning. This diversity is depicted along with the most recent phylogenetic hypothesis for this genus (Zink *et al.*, 1999). The full and open circles at the tips of this phylogeny represent species that develop fully vascularised male brood patches or sometimes develop partially developed brood patches respectively. There are no data on male brood patch vascularisation in *Toxostoma cinereum*. Voucher numbers for eggs photographed at the American Museum of Natural History (AMNH): *Oreoscoptes montanus,* EN-3579; *Toxostoma redivivum,* EN-2959; *T. lecontei,* EN-17400; *T. crissale,* EN-3576; *T. bendirei,* EN-11867; *T. cinereum,* EN-3584; *T. curvirostre,* EN-2938; *T. rufum,* EN-11819; *T. longirostre,* EN-2917.

colouration and patterning of their eggs (Figure 2), and also show variation in male brood patch development. First, I examine whether blue-green eggshell chroma is positively, and brown eggshell chroma negatively, related to eggshell conspicuous with respect to an avian viewer. I expect that this quantitative approach will validate qualitative assessments that suggest blue-green eggs are conspicuous and brown eggs are cryptic. Second, I compare the variances in eggshell conspicuousness across these generated ranges, to determine if there were differences in means and variances or just mean trait values. Here I expect differences in variance between blue and brown eggs. Third, I will determine if effect sizes of evolutionary models increase as they focus on more conspicuous trait values, and I will determine if eggshell conspicuousness is higher in species where males possess a fully vascularised brood patch. I expect that species which develop full male brood patch vascularisation will have more conspicuous eggshells than species where males do not form fully developed brood patches. I also expect that the effect of these models will increase when examining the more conspicuous tail of these populations. The blackmail hypothesis predicts that the ultimate cause for this relationship is selection on males to keep their eggs concealed. To assess the critical assumption that male brood patch development is related to the time they spend covering their eggs, I examine this relationship across more than 300 North American breeding birds.

2. METHODS

2.1 Taxon sampling

The American thrashers (*Toxostoma spp.*) exhibit dramatic variation in egg colouration and comprise a small monophyly of ten species (Zink *et al.*, 1999) (Figure 2). Male incubation is a trait common across *Oreoscoptes* and *Toxostoma* (Bent, 1948; Verner and Willson, 1969). To assess paternal care associated with incubation, I used information on the degree of male brood patch vascularisation, which in *Toxostoma* varies from no brood patch to a partial – nearly complete brood patch (Pyle, 1997). The values and data in this publication (Pyle, 1997) are essential for sexing and aging North American birds in the field, and was the product of peer-reviewed literature, as well as historic banding records (Pyle, 1997). I recreated a phylogeny using data from a current hypothesis on the relationships among *Toxostoma* (Zink *et al.*, 1999), using the program Mesquite (version 2.6).

However, male brood patch vascularisation may not be an adequate proxy for paternal investment. Therefore, I validated this assumption by examining paternal effort and brood patch development across a large number of North American breeding birds. I used the Birds of North America to determine relative male incubation of 311 species, which I classified as no incubation = 0, occasional incubation (or < 5%) = 1, moderately low incubation (< 30%) = 2, moderately high incubation (< 50%) = 3, equal to female effort = 4, or more than the female = 5. I chose this metric rather than percent attendance, because these data could be obtained more reliably from species accounts, were more comparable, and less prone to error. I then classified male brood patch vascularisation for each species in Peter Pyle's Identification Guide (Pyle, 1997), and checked these using separate species accounts from the Birds of North America. The classifications were: N, for no brood patch vascularisation, P3, for very sparse defeathering giving the impression of a brood patch in some males, P2, for species where some males can develop an incomplete brood patch, P1, for species where males can develop a partial brood patch, P for species where males do develop partial brood patches, and Y to represent species where males develop fully vascularised brood patches.

I then used a large phylogeny for all of Aves from the Tree of Life Web project (Maddison and Schulz, 2007), and replaced all Passeri with a 1723 tip oscine supertree (Jønsson and Fjeldså, 2006). I used TreeSnatcher (Laubach and Haeseler, 2007) to transcribe the Passeri supertree, and TreeGraph2 (Stöver and Müller, 2010) to merge the two phylogenies. These trees are based on molecular evidence and are all carefully proof-read and up-to-date. I then truncated this large phylogeny to include only species for which I had documented male brood patch vascularisation.

2.2 Egg colour quantification

I measured 161 eggs, from 52 egg sets of nine species (*Toxostoma bendirei, T. cinerium, T. crissale, T. curvirostre, T. lecontei, T. longirostre, T. redivivium, T. rufum, Oreoscoptes montanus*) using a reflectance spectrometer (Ocean Optics USB4000) with a portable light source (Ocean Optics PX-2 pulsed xenon light source), and an angled probe tip (45 degrees) to avoid specular glare. These eggs were stored at the American Museum of Natural History, the Field Museum of Natural History, and University of Michigan's Museum of Zoology. I averaged two spectrometer readings from the lower, medial, and upper portion of each eggshell to obtain an overall eggshell reflectance and these spectra were used to generate an average spectral reflectance for each species (for further details see Hanley *et al.*, 2012). Colour in these eggs varied from blue-green to heavily speckled and almost completely brown (Figure 2). In every case the size of egg speckles was smaller than the measurement area, and therefore their reflectance spectra were integrated into the background spectra. This would result in artificially high conspicuousness values for the more cryptic speckled eggs (Hanley *et al.*, in press), making the results presented here conservative. In addition, I also measured nest colouration from nests of *T. bendirei* (N =1), *T. crissale* (N =1), *T. redivivium* (N =1), *T. rufum* (N =2) in a similar way although I used a 90 degree incidence angle and sampled five readings from the centre and four evenly spaced positions along the sides of each nest cup.

For colour analysis, I restricted these analyses to blue-green and brown colouration because these are the colours produced through the two main pigment classes found in avian eggs (Kennedy and Vevers, 1976). I present blue-green chroma as the proportion of reflectance in the blue-green portion of the spectrum (450–550 nm) relative to the entire spectrum (300–700 nm), and brown chroma as the proportion of reflectance in the red portion of the spectrum (600–700 nm) relative to the entire spectrum (300–700 nm). Previous research has found that blue-green chroma is a good approximation of biliverdin content in eggshells (Lopez-Rull *et al.*, 2008; Moreno *et al.*, 2006)

To create variation in potential eggshell conspicuousness, I calculated nine intermediate nest spectra from within the range of nest backgrounds measured (the nest of *T. bendirei* being darkest, and the nest of *T. crissale* being brightest). To create this range, I multiplied the difference at each wavelength between the brightest nest background and the darkest nest background by a constant. This incremental difference was added to the darkest nest, and was repeated by increasing the constant nine times to result in nine intermediate nest backgrounds (Figure 3). These interpolated backgrounds were created using the following function

$$R_{\beta j} = C_j * (R_{d\lambda i} - R_{l\lambda i}) + R_{d\lambda i} \tag{1}$$

Where $R_{d\lambda i}$ is the reflectance at wavelength *i* for the dark background, $R_{l\lambda i}$ is the reflectance at wavelength *i* for the light background, and C is a constant between 0 and 1 for each of the *j* interpolated backgrounds. This range of potential background spectra was used to generate a range of theoretical eggshell conspicuousness values.

2.3 Avian visual modelling

I used a receptor noise-limited opponent model using pavo: an R Package for Analysis, Visualization and Organization of Spectral Data (Maia *et al.*, submitted)

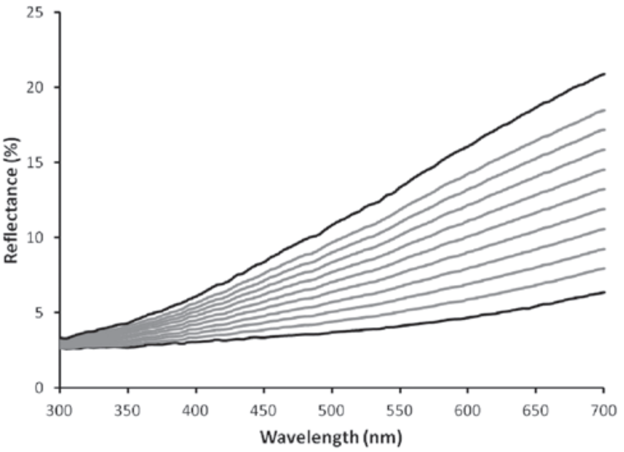

Figure 3 The spectral reflectance (%) generated to create a range of eggshell conspicuousness values for each species. Here the bottom dark line represents the reflectance for an average *Toxostoma bendirei*, while the top dark line represents the spectral reflectance of a *Toxostoma crissale* nest, which were the darkest and lightest American thrasher nests measured respectively. The grey lines represent the interpolated nest backgrounds generated as intermediate to these extreme measurements. This effectively created a range of values to compare eggs against that resulted in a range of eggshell conspicuousness values (*see Methods for more details*).

in the statistical package R (R Development Core Team, 2010) to determine the discriminibility (Vorobyev and Osorio, 1998; Vorobyev *et al.*, 1998), Δ*S*, between the quantum catches of the nests and eggs and I will refer to these scores as 'conspicuousness'. Since information on thrasher cone abundance was not available, I used cone spectral sensitivity for an average UVS bird (Endler and Mielke, 2005), media transmission (scaled to one), and relative cone abundance values for the blackbird (*Turdus merula*) (Hart *et al.*, 2000) to determine the receptor noise and quantum catch for each receptor type. These calculations depend on a scaling factor relating to intensity of illumination (Vorobyev, 2003), and a Weber fraction that account for receptor noise relating to signal intensity and photon catch. Most previous research employing these methods use high light intensities and a set Weber fraction, and these, therefore, are the default settings for some software (Hadfield, 2004); however, the best values for these factors are not known for birds and the influence of incorrect choices on our estimates of conspicuousness are also unclear.

I modelled the influence of both of these factors on conspicuousness estimates to examine the influence of these factors and to choose values that would introduce the least bias. For this I calculated the conspicuousness of eggs of every thrasher species each against all nests (real and interpolated), by choosing 50 values for both the scaling factor and the Weber fraction that were beyond their accepted ranges, 1 to 50,000 and 0.01 to 2.0 respectively (Figure 4), resulting in 2,500 separate visual models for every egg–nest combination, hence 270,000 separate visual models in total. While making the appropriate choices for these factors is impossible without behaviour experimentation (lacking in nearly every study using this method), it is possible to choose values that produce the most consistent results. For each egg–nest pairing I subtracted the mean conspicuousness score for each Weber fraction or scaling factor setting from the global median (to account for extreme outliers in the 2,500 visual models; Figures 4B and C). The minimum absolute value of this estimator indicates the settings that introduced the least bias into the conspicuousness scores of every egg–nest combination. Then I used the average of these estimators as the scaling factor (8973.22) and the Weber fraction (0.11 for all photoreceptors) for further analyses. These values should produce light and Weber fraction invariant conspicuousness estimates. This suggests that previous findings would also be robust despite slight estimation error for these factors.

Perceived differences in colour also depend on the light environment, therefore I considered the irradiance spectra of multiple light environments (D65 standard illumination representing daylight conditions, forest shade, and blue sky light) (Endler, 1993). A similar approach found that these differences in irradiance spectra did not influence the conspicuousness of cuckoo eggs from host eggs, or cuckoo or host eggs from host nests (Langmore *et al.*, 2009), and therefore I expect that these three irradiance spectra will produce similar results. In addition to modelling cone capture, I also examined the influence of luminance discrimination (*for equations see* Hanley, *et al.*, 2013; Siddiqi *et al.*, 2004). The double cones are believed to be responsible for this ability to discriminate differences in brightness (Jones and Osorio, 2004), and this may be important for birds. Here I expect that luminance discrimination will not be related to eggshell colorimetric descriptors or brood patch development, because in contrast to blue-green chroma, relatively less variation is attributed to brightness differences between species within the same genus (Hanley *et al.*, 2012). In other words, while there are large interspecific differences in eggshell hue (blue-green, brown, or white), eggs from any species may be relatively light or dark. Moreover, if these colorimetric variables are useful colour descriptors they should describe chromatic better than achromatic variation in colour.

2.4 Comparative analysis

When examining the relationship between organisms, it is important to not consider species as independent data points, and the phylogenetic generalised least squares (PGLS) analysis controls for this phylogenetic co-variation (Freckleton *et al.*, 2002; Pagel, 1997, 1999). For all analyses I used PGLS regressions using the package APE (Paradis *et al.*, 2004) in the statistical package R (R Development Core Team, 2010) to control for the

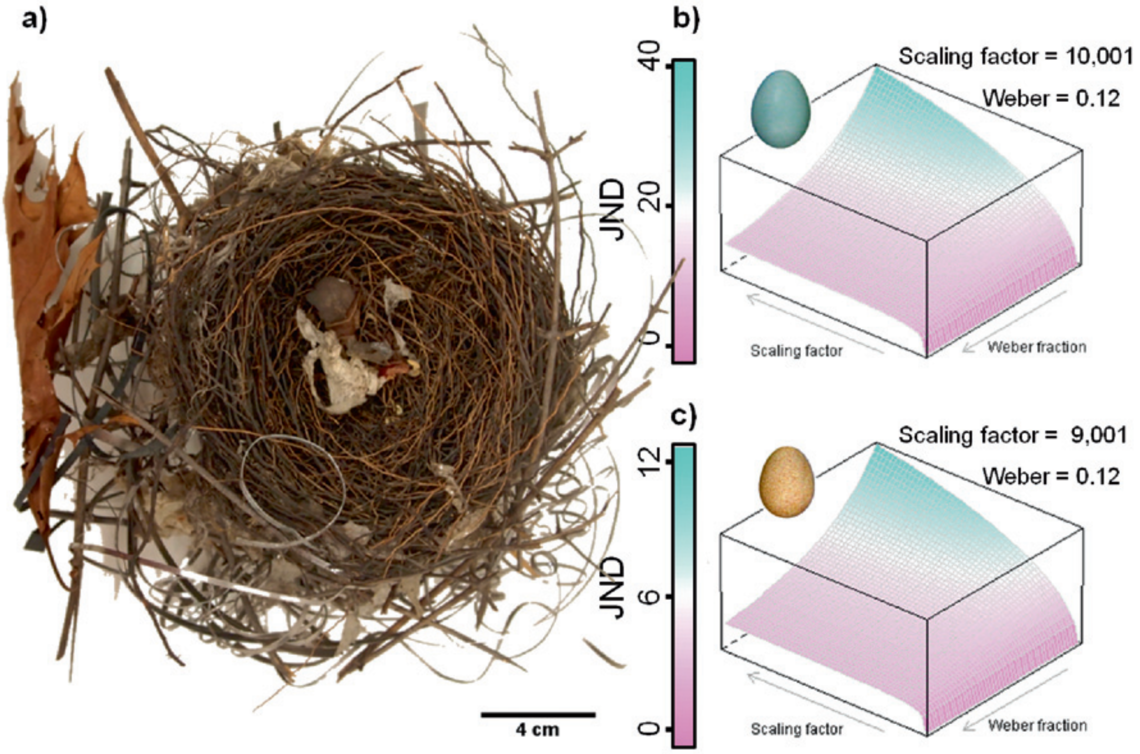

Figure 4 Receptor noise-limited models are useful tools to understanding visual perception and signalling in animals, but their ability depends on a variety of assumptions that may impact the accuracy and reproducibility of their results. (a) Nest of a brown thrasher, *Toxostoma rufum,* compared to a (b) Crissal's thrasher, *Toxostoma crissale* egg, and a (c) brown thrasher egg. For these comparisons I produce conspicuousness scores (in JND units) by varying the Weber fraction and the light intensity scaling factor. This produced 2,700 separate estimates for each comparison, and those are depicted in two surface plots (b) and (c). These visual models combine both noise in quanta catch as well as neural processing. Under low illumination noise should roughly correspond to the square root of light intensity, while under high illumination noise is independent of light intensity and should follow Weber's law. When the Weber fraction is low the modelled noise is predominantly due to intensity, and as the Weber fraction increases its influence on noise also increases. Therefore, variation in the parameters used will results in different estimates of conspicuousness that are the product of variable combinations of these two types of noise. Importantly, while changing these parameter values will produce different estimates of conspicuousness for any egg to nest comparison [pink to blue in (b) and (c)], when choosing consistent parameter values the relative differences in conspicuousness between species should remain constant, because the shape of this surface is dictated by the receptor noise-limited model not any particular egg or nest.

degree of phylogenetic relationships among species. For our PGLS analyses we used the maximum likelihood value of Pagel's λ (Pagel 1997, 1999), which transforms a phylogeny according to the best fit of the data to a Brownian motion model of evolution (Freckleton *et al.*, 2002) . To help evaluate biological effect I present Hedges' *d*, which corrects Hedges' *g* (or Cohen's *d*) for bias due to small sample sizes (Hedges, 1981; Hedges and Olkin, 1985; Nakagawa and Cuthill, 2007) and I present exact confidence intervals for this effect estimate (Nakagawa and Cuthill, 2007). I refer to this measure of effect as d_{unbiased}, following (Nakagawa and Cuthill, 2007).

2.5 Are blue-green thrasher eggs conspicuous?

I ran PGLS analyses to determine if the colorimetric variables used to describe eggshell colour predict eggshell conspicuousness and luminance discrimination. In these models, either eggshell conspicuousness or eggshell luminance contrast were the dependent variables and the colorimetric variable were the predictors. Since I calculated a range of eggshell conspicuousness values, I repeated these models each time predicting a different species-specific contrast score (either chromatic or achromatic). Each species-specific contrast score was calculated against a different potential background (dark, interpolated, and light), such that each species had contrast score values associated with relatively cryptic eggs for their species through relatively conspicuous eggs for their species. This produced 12 models ranging from relatively cryptic eggs (in this case placed against a relatively dark background) to relatively conspicuous eggs (in this case, placed upon a relative light background) for each species. To determine if these patterns are consistent across a variety of light conditions, I repeated these models under multiple light environments and using an unbiased scaling factor as well as scaling factors generally accepted as representing dark (500) and bright illumination (10,000) conditions. Because these colour metrics describe the chromatic colour signal I expect that these will directly relate to conspicuousness but will be unrelated to luminance discrimination.

2.6 Is it better to focus on mean values or extreme values?

To determine if we should expect that species should have similar means but different variances, I randomly generated normal distributions ($N = 100{,}000$) of eggshell conspicuousness values using means and standard deviations for each species (Figure 1E) in R (R Development Core Team, 2010). This effectively created a larger 'population' based on the range of data. The variances of these populations were compared with F tests (Faraway, 2005). I report the ratio of the sample variances for each species pairing, as well as the associated confidence intervals and *P* values.

2.7 Have thrashers evolved brood patches in response to conspicuousness?

For each light environment, I ran PGLS analyses to determine if there was a difference in eggshell conspicuousness between thrasher species that exhibit male brood patches, and those that do not. In these models eggshell conspicuousness was the dependent variable and the degree of male brood patch vascularisation was the predictor. To determine how these results were influenced by choosing relatively conspicuous versus relative cryptic eggs for each species, I reran these models each time including a different species-specific conspicuousness or luminance discrimination score (both measured in just noticeable difference, or JND, units). I then preformed a likelihood ratio test to determine if male brood patch vascularisation provided a better fit to the model predicting either relatively high eggshell conspicuousness or the model predicting relatively low eggshell conspicuousness. These tests were run across multiple light environments to determine how varying light conditions may influence these findings.

In addition, I also examined the relationship between male brood patch vascularisation and male nest attendance across birds from 32 families to test the prediction that male brood patches reveal information about nest attendance rates. For this I ran a similar PGLS model predicting relative paternal effort by degree of male brood patch vascularisation. I also examined this relationship with a standard ANOVA for which a lager sample size was available.

3. RESULTS

3.1 Are blue-green thrasher eggs conspicuous?

It is important to address if the colorimetric variables assessed from eggshell reflectance measures correspond with their conspicuousness to avian viewers. As expected, I found that blue-green chroma was positively related to conspicuousness in all light environments and light intensities, except when eggs are viewed against light nests under blue sky light (Figure 5A–C). In contrast, brown chroma exhibited the opposite pattern; however, in all light environments this relationship was non-significant for the darkest nests (Figure 5D–F). Interestingly, these patterns

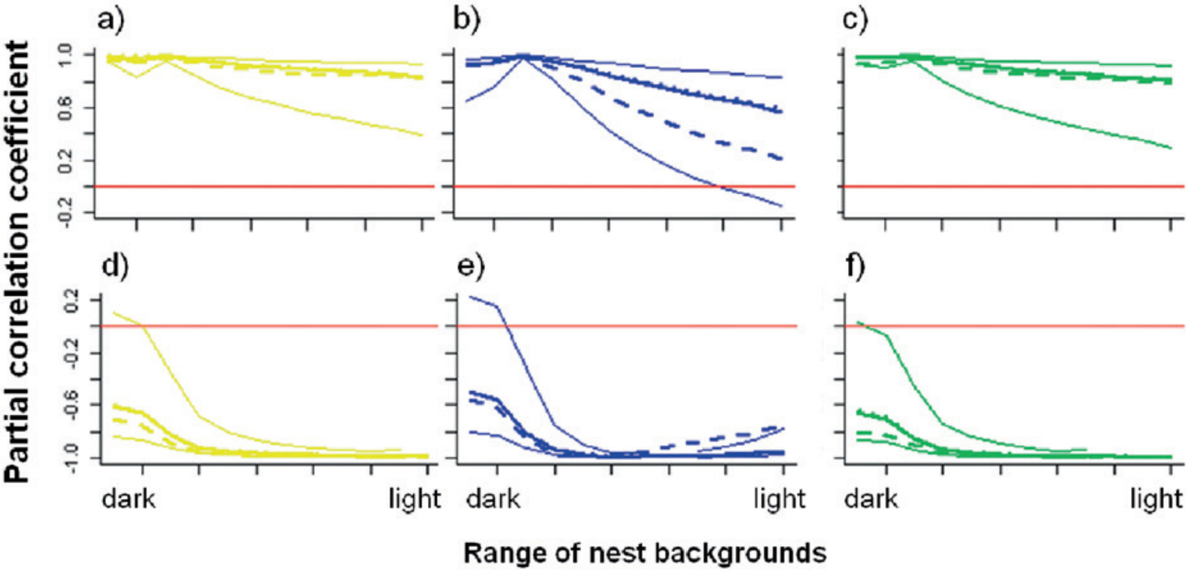

Figure 5 Results from sequential phylogenetic least squares analyses relating blue-green (a)–(c) and brown chroma (d)–(f) to calculated conspicuousness under average daylight (yellow), blue-sky light (blue), and forest shade (green). Lines represent conspicuousness calculated using least biased parameters (solid –see Methods for details), high light intensity (dotted), or low light intensity (dashed). Thin solid lines represent the exact confidence intervals around the conspicuousness estimates calculated using the least biased parameters. Confidence intervals (95%) overlapping zero (red line) represent tests that would be non-significant at the 0.05 level.

were generally consistent across all light intensities except under blue sky illumination at low intensities (Figure 5B), which is an unrealistic combination for an open nesting bird. Therefore, blue-green eggs viewed by a UV sensitive bird are more likely to be visually detected in most nests, light intensities, and under most light conditions. As expected, neither blue-green chroma nor brown chroma was related to luminance discrimination in any light environment or with any parameter values (all $P > 0.19$ and 0.45, respectively).

3.2 Is it better to focus on mean values or extreme values?

The variance in eggshell conspicuousness between these species differed significantly (Table 1), despite having similar means (Figure 1E). Therefore, applying tests across a range of trait values was warranted.

3.3 Have thrashers evolved brood patches in response to conspicuousness?

Eggshell conspicuousness was greatest in species with a high degree of male brood patch vascularisation in all ambient light conditions (Figure 6). This pattern was significant across the entire conspicuousness range, except when using the most cryptic eggs. Moreover, these effect sizes were large considering the limited sample size. In addition, these effects were consistent between light environments (Figure 6), and the relationship between eggshell conspicuousness and male brood patch development strengthened when models were run on subsets of the data that represented more conspicuous eggshells (Figure 6). Altering light intensity, and using a Weber fraction of 0.05 for all cones, produced similar results (unpublished data). Likelihood ratio tests found that the models run on the most conspicuous eggs provided a significantly better fit than similar models run on the most cryptic eggs in daylight ($\chi = 11.01$, $P < 0.001$), blue skylight ($\chi = 13.15$, $P < 0.001$), or forest shade ($\chi = 9.84$, $P = 0.002$), when receptor-noise limited models were based on least biased parameters. However, in contrast to these findings with conspicuousness, luminance discrimination under average daylight, blue sky light, and forest shade was unrelated to male brood patch development, regardless of the light intensity (all $P > 0.61$, 0.53, and 0.27 respectively). Differences in degree of brood patch vascularisation likely translate into differences in paternal effort, because male incubation effect was significantly predicted by the extent of brood patch vascularisation across 32 avian families ($F_{5,214} = 47.05$, $P < 0.0001$). Here I found that North American species with more developed male brood patches had greater relative male incubation. To account for species for which phylogenetic information was lacking, I conducted a similar non-phylogenetic ANOVA including data from 36 avian families found the same pattern ($F_{5,305} = 200.4$, $P < 0.0001$; Figure 7).

Table 1 For each species a new sample of 100,000 eggshell conspicuousness values were generated randomly in R (R Development Core Team, 2010) using that species' mean and 95% confidence intervals. The variances of those samples were compared using an F test. The ratio of sample variances between species (lower $CI_{0.95}$, upper $CI_{0.95}$) is shown in the upper triangle, and the P values from the test in the lower triangle

	O. montanus	***T. longirostris***	***T. benderei***	***T. cinerei***	***T. crissale***	***T. curvirostris***	***T. lecontei***	***T. redivivum***	***T. rufum***
O. montanus		0.18 (0.18, 0.19)	5.67 (5.6, 5.74)	5.18 (5.11, 5.24)	2.28 (2.26, 2.31)	3.35 (3.3, 3.39)	5.63 (5.56, 5.7)	2.02 (2, 2.05)	1.26 (1.25, 1.28)
T. longirostris	< 0.0001		1.04 (1.03, 1.06)	0.95 (0.94, 0.97)	0.42 (0.42, 0.43)	0.62 (0.61, 0.62)	1.04 (1.02, 1.05)	0.37 (0.37, 0.38)	0.23 (0.23, 0.24)
T. benderei	< 0.0001	< 0.0001		0.91 (0.9, 0.93)	0.4 (0.4, 0.41)	0.59 (0.58, 0.6)	0.99 (0.98, 1.01)	0.36 (0.35, 0.36)	0.22 (0.22, 0.23)
T. cinerei	< 0.0001	< 0.0001	< 0.0001		0.44 (0.44, 0.45)	0.65 (0.64, 0.65)	1.09 (1.07, 1.1)	0.39 (0.39, 0.4)	0.24 (0.24, 0.25)
T. crissale	< 0.0001	< 0.0001	< 0.0001	< 0.0001		1.46 (1.45, 1.48)	2.46 (2.43, 2.49)	0.88 (0.87, 0.9)	0.55 (0.55, 0.56)
T. curvirostris	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		1.68 (1.66, 1.7)	0.6 (0.6, 0.61)	0.38 (0.37, 0.38)
T. lecontei	< 0.0001	< 0.0001	0.31	< 0.0001	< 0.0001	< 0.0001		0.36 (0.35, 0.36)	0.22 (0.22, 0.23)
T. redivivum	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.62 (0.62, 0.63)
T. rufum	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

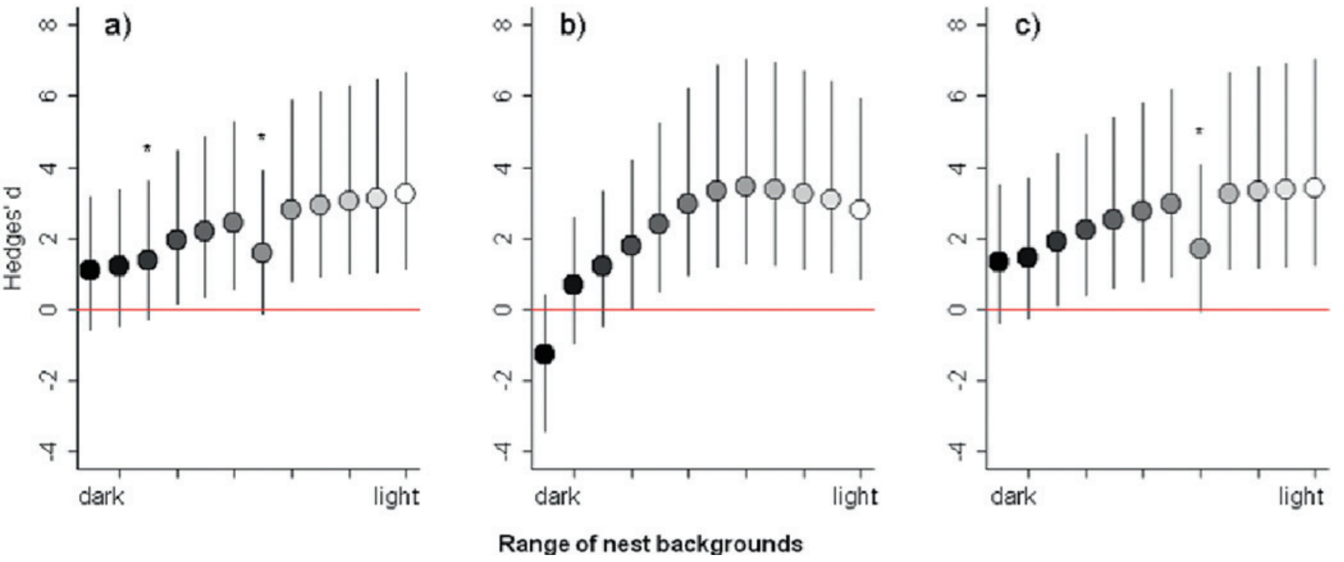

Figure 6 Hedge's *d* effect measures with exact confidence intervals, from phylogenetic least squares (PGLS) analyses predicting just noticeable differences by male brood patch vascularisation across the American Thrashers in (a) daylight, (b) blue-sky light, and (c) forest shade illumination. Under each light condition, 12 PGLS analyses ran across a range of generated eggshell conspicuousness values from dark nests representing the lowest average eggshell conspicuousness (dark filled circles), to light nests representing the most conspicuous eggshells across species (light filled circles). Italics represent models where Pagel's lambda was negative but needed to be fixed to zero to generate model output. The horizontal red line represents an effect size of zero. Confidence intervals (95%) that overlap zero represent test that would not be significant at the 0.05 level.

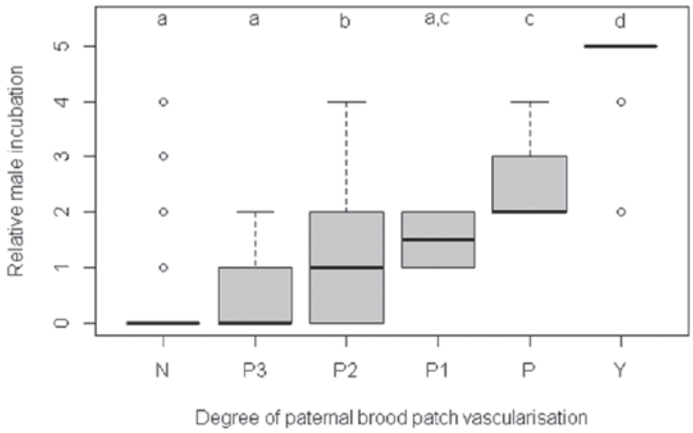

Figure 7 The relationship between relative paternal incubation effort and degree of paternal brood patch vascularisation across 36 avian families. A relative paternal incubation effort score of 0 represents no incubation, a score of 1 indicates occasional incubation (or < 5%), a score of 2 indicates moderately low incubation (< 30%), a score of 3 indicates moderately high incubation (< 50%), a score of 4 indicates that males and female contribute equally, and a score of 5 indicates that the male contributes relatively more effort that the female. Male brood patch vascularisation was classified as N = no brood patch vascularisation, P3 = very sparse defeathering giving the impression of a brood patch in some males, P2 = some males can develop an incomplete brood patch, P1 = males can develop a partial brood patch but probably less extensive than the female, P = males do develop partial brood patches, and Y = males develop fully vascularised brood patches. The letters above bars represent post-hoc significance values from a non-phylogenetic ANOVA.

4. DISCUSSION

The American thrashers exhibit a dramatic degree of variation in eggshell colouration and patterning over a small monophyly. Here, I show that blue-green chroma increases, while brown chroma decreases conspicuousness of thrasher eggs to an avian viewer under several light conditions and across most nest types. I also found that eggshell conspicuousness was greater in thrasher species where males develop brood patches. A comparative analysis including more than 300 North American breeding birds suggests that male brood patch vascularisation directly relates to paternal effort during the incubation period. Interestingly, within the American thrashers, this positive relationship between eggshell conspicuousness and male brood patch vascularisation strengthens when examining increasingly conspicuous eggshell values.

Examining patterns across a range of background spectra recognises greater potential sample variation, and allows us to examine how systematically altering a trait influences results and interpretation. The generality of common comparative analyses would often overlook this within-species variation in conspicuousness. This oversight of within-species variation has been recently criticised (Garamszegi and Møller, 2010) and alternative analytical methods have been proposed to incorporate within- and between-species variation (Hadfield and Nakagawa, 2010); however, examination of mean differences still predominates comparative approaches. While controlling for between-species covariation as well as within-species trait variation is ideal, extensive within-species data are lacking in many cases. Instead, to examine the relationship between egg conspicuousness and the degree of male brood patch vascularisation, I have run multiple separate analyses focusing on theoretical subsets of populations ranging from those least conspicuous to most conspicuous. Such intraspecific or interspecific variation in nest material may be expected if parents constructing nests need to balance nest construction with other activities (Moreno *et al.*, 2008; Mountjoy and Robertson, 1988), or if competition exists over nest materials (Skutch, 1976). As selection should not act equally on cryptic and conspicuous eggs within or between species, such segregation seems reasonable.

The relationship between male brood patch vascularisation and eggshell conspicuousness was non-significant when examining relatively cryptic eggs among thrasher species; however, the effect size rapidly increased when examining progressively more conspicuous eggs (Figure 6). This suggests that males from species with greater eggshell conspicuousness have more fully developed brood patches. Moreover, this pattern holds across a range of conspicuousness values and generally increases when the most conspicuous eggs from each species are compared. With the exception of three species (*Toxostoma guttatum, T. ocellatum, T. arenicola*) for which eggs were unavailable, this represents the entirety of the genus. Therefore, in this case low sample size was not the result of sampling effort, but reflects the fact that there are relatively few thrasher species. Despite these low sample sizes the majority of these findings were significant. However, measures of statistical effect with exact confidence intervals around those effect measures are more meaningful when interpreting biological significance than *P* values (Nakagawa and Cuthill, 2007), particularly when sample size is so restricted. In this case, I found strong effect sizes that were generally consistent between three light environments.

These findings support an assumption and a prediction of a recently proposed hypothesis that suggests conspicuous egg colouration evolved through sexual conflict between the mated pair, eliciting more care from the male to keep eggs covered (Hanley *et al.*, 2010). First, these results show that blue-green egg colour increases risk of avian visual detection, while brown chroma (*i.e.*, speckling) serves to make these eggs less conspicuous, which has important implications concerning predation and brood parasitism risk. A review on egg colouration has shown that blue-green eggs are more common in open nesting species (Kilner, 2006), which is a curious finding if these eggs are more obvious to avian predators and brood parasites. Second, eggshell conspicuousness is related to the degree of paternal brood patch vascularisation in American thrashers. I suggest this pattern results from selection pressures to conceal conspicuous eggs from avian predators and brood parasites. While no data exist on how male brood patch vascularisation relates to incubation attendance in American thrashers, a broad comparative analysis found that the degree of male brood patch vascularisation was positively associated with male nest attendance in the incubation period. This evidence supports the prediction that male thrashers are incubating conspicuous eggs more than they are cryptic eggs.

These relationships between colour metrics and conspicuousness estimates corroborate general qualitative assessments of eggshell colouration. This pattern was strong in all light environments and nest types, except for the lightest (for blue-green chroma) and darkest nests (for brown chroma), and when eggs are compared to light nest material under blue sky conditions at low light levels. This finding is reasonable because colour discrimination weakens at low light levels (Wyszceki and Stiles, 1982; Vorobyev and Osorio, 1998; Cassey, 2009), and these low light intensities are among the lowest values possible during normal daylight conditions (Vorobyev, 2003). Moreover, these models do not adequately predict bird or human vision under low illumination (Vorobyev and Osorio, 1998), and birds require at least 10 times more light than humans for colour discrimination (Kelber and Lind, 2010). It is important to note that these comparisons were made when all variables (*e.g.*, light level and nest colour) were consistent between the clutches being compared. Although this proves that blue-green eggs are generally more conspicuous, ultimately the conspicuousness of the clutch depends on the materials available for nest construction and the light environment the nest is placed in. While this fact is already understood from the perspective of visual modelling (Bennett *et al.*, 1994; Endler, 1990), these results quantitatively illustrate how ignoring the light environment and nesting substrate may impact egg colour research. Variation in the colour of available and chosen nest material probably varies between individuals, as well as within and between populations and communities. Therefore, accounting for variation in nest colour may be important, especially because recent research suggests that the similarity between nest contents and nest material is related to predation rate (Mayer *et al.*, 2009).

Recent evidence suggests that biliverdin is physiologically expensive (Morales *et al.*, 2006; Morales *et al.*, 2008); therefore, the persistence of this trait and the relationship between eggshell conspicuousness and male brood patch vascularisation may be maintained by sexual selection as proposed by the sexual signalling hypothesis (Moreno and Osorno, 2003). For example, fully vascularised male brood patches may develop in birds where females lay blue-green eggs that allow for the assessment of female quality. Therefore, these findings could also lend support to the growing body of literature on how blue-green eggshell chroma influences paternal care (Cherry and Gosler, 2010; Reynolds *et al.*, 2009). Although the sexual signalling and blackmail hypotheses are not mutually exclusive, it is unlikely that these mechanisms originated simultaneously. If males used biliverdin as a cue for assessing female quality, reduced visibility of the nest contents caused by male incubation may be a beneficial consequence of the signal, freeing eggs from constraints of visual detection and allowing for the elaboration of a signal of female quality. However, it seems more parsimonious to assume that a mechanism for clutch protection evolved first, and then subsequently pigment levels increased allowing for enough variation in blue-green colour for inter-female comparisons. Future research should investigate the origin of these two distinct mechanisms.

In addition, future research should also investigate the role males play in nest material selection and nest construction. Nest building behaviour is not well known for most thrasher species and it is possible that males could use this form of parental investment as a counter strategy. For example, dark nest materials reduced conspicuousness in this study and may also reduce the conspicuousness of mates' eggs. By reducing the risk associated with conspicuous eggshells, males may reduce the possibility for coercion induced by blackmail. It will also be important to examine the predictive ability of avian visual models under practical, natural settings. While these models do provide great insight, the assumptions of these models are rarely investigated, and their results are rarely corroborated with behavioural experimentation. Here I used parameters that produced the least biased model output; however, similar to the majority of other studies using this technique, the choices are somewhat arbitrary. Nonetheless, the least biased estimates for the light intensity scaling factor were within the normal daylight range (Vorobyev, 2003) and the results were robust across various light intensities, types of illumination, and across a range of trait values. In addition, the choice of Weber fraction should not influence the relative difference between eggs and nests (Langmore *et al.*, 2009) and these results the same when a Weber fraction of 0.05 was used (*unpublished data, DH*). This suggests that although the model inputs may be refined by future behavioural experimentation, it is unlikely that other values would alter the interpretation.

I acknowledge that I was limited in the number and age of both nests and eggs used in this study, and I also realise that the materials that I used in this study could have faded. However, these nests have been kept out of the light for much of their tenure, and are constructed of dead twigs and branches. The reflectance spectra of these nests were spectrally similar across species, and it isn't likely that some nest materials fade significantly more than others. In addition, the nest reflectance values were simply used to create a realistic range of eggshell conspicuousness values. If active nests are more variable, the true range of eggshell conspicuousness values was probably larger than depicted here. However, these results show a consistent pattern and would suggest that an increased range would only enhance the existing patterns (Figure 6). In addition, it is possible that the eggshells I sampled may have faded (Cassey *et al.*, 2010), though I suggest that this has not influenced these results in such a way as to produce the reported patterns (for further details see Hanley *et al.*, 2012). First, these eggs were all of a similar age (mean ± SD = 1904 ± 13.8), and thus subject to similar fading. Second, these eggs did not appear faded and exhibited a gradient of colour from brown to blue-green (Figure 2). Third, any fading would only have decreased the range of conspicuousness values and statistical power; because the less faded fresh eggs would have had greater chroma values, which were directly related to conspicuousness values.

This study provides evidence that in the American thrashers, male brood patch vascularisation is greatest in species with conspicuous eggs. Although incubation attendance data are not available for American thrashers, a comparative analysis including more than 300 species shows that males developing brood patches provide greater incubation attendance. Together, these findings suggest that males may be morphologically and behaviourally adapted to mitigate the visual risk of conspicuous eggs. It is possible that the fitness benefits associated with successfully blocking visual detection of conspicuous nest contents may compensate for thermal costs associated with contact incubation (Deeming, 2008), or costs of predation and parasitism (Hanley *et al.*, 2012). Future work would benefit from considering visual risk and thermal demands as synergistic pressures when examining the costs and benefits of incubation behaviour in birds. Interestingly, the relationship between eggshell conspicuousness and male brood patch vascularisation strengthens when examining the most conspicuous individuals for each species. These conspicuous individuals theoretically experience the greatest selection pressure; therefore, these findings suggest the importance of examining both intra- and inter-species variation, and examining the pressures experienced by extreme phenotypes. To my knowledge, this is the first study to document that egg colouration is related to brood patch vascularisation and to show that the degree of male brood patch vascularisation is directly proportional to relative paternal incubation effort in birds.

ACKNOWLEDGEMENTS

I thank J. Hinshaw, D. Willard, P. Sweet, and P. Capainolo for assistance in their collections. I also thank J. L. Cuthbert, G. Betini, D. LaClair, T. D. Flockhart, G. Mitchell and D. R. Norris for their helpful comments on this project. I also thank three anonymous reviewers and Dr T. Sands for their helpful comments on this manuscript. I am especially thankful to S.M. Doucet who helped refine my thoughts on eggshell colour evolution and who provided equipment necessary for this project. In addition, I would like to thank the Mitacs Elevate Postdoctoral Fellowship program, the Frank M. Chapman fund, the American Museum of Natural History and Field Museum of Natural History for financial support, and the University of

Guelph for use of their facilities. When participating in this work DH was co-financed by the European Social Fund and the state budget of the Czech Republic (project no. CZ.1.07/2.3.00/30.0041).

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