

Are avian eggshell colours effective intraspecific communication signals in the Muscicapoidea? A perceptual modelling approach

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Diversity in the colour and appearance of avian eggshells has been proposed to serve a variety of visual functions, including crypsis from predation, mimicry and discrimination in facultative and obligate brood parasitism, and sexually selected intraspecific signalling of the extent of maternal investment in the egg. Here, we apply a photoreceptor noise-limited colour opponent model of avian perception to assess a necessary corollary of any intraspecific signalling hypothesis, namely that individual birds are able to discriminate between colours of eggs in different conspecific clutches. Clutches from 46 species in the superfamily Muscicapoidea were measured at the Natural History Museum collection in Tring, UK. The results demonstrate that, for these particular species, most eggs are predicted not to be easily discriminable from those in other conspecific clutches in terms of the shells' background coloration. These findings are of fundamental concern to any signalling hypothesis that looks to explain the evolution of avian-visible egg colour polymorphism through selection at the intraspecific level. Importantly, future studies should combine both the proximate mechanisms and the ultimate functions of trait variability when testing hypotheses of the variability in eggshell appearance.

Keywords: communication, sexual selection, signals, visual perception.

Considerable variation exists in the appearance of avian eggshells (Walters 2006), and this variation has long impressed evolutionary biologists (Wallace 1889, Lack 1958). Eggshell pigments have been hypothesized to support various physical roles, including strengthening the shell matrix (Solomon 1997, Gosler *et al.* 2005) and/or filtering harmful sunlight radiation (Bakken *et al.* 1978, Lahti 2008). Alternatively, differences in eggshell appearance may be trade-offs between physical benefits and costs and a variety of signalling/communication roles (Magige *et al.* 2008). These include egg recognition in colonial species (Birkhead 1978, Gaston *et al.* 1993), the detection of intra- and interspecific brood parasitism (Davies 2000, Lyon

2003), and the avoidance of predation through either crypsis or aposematism (reviewed in Underwood & Sealy 2002, Kilner 2006).

To date, no single explanation of visual signalling alone has explained fully the extent of colour diversity across all, or even most, avian families (Kilner 2006). Many species that lay colourful and maculated eggs are neither colonial nor known hosts of intra- or interspecific brood parasites (Underwood & Sealy 2002). Similarly, a number of experimental tests have failed to support the antipredator hypothesis for eggshell coloration (Götmark 1992, Westmoreland & Kiltie 1996, Weidinger 2001), and it is likely that differences in nest architecture are more important than egg colours in concealing clutches from predators (Weidinger 2004; but see Westmoreland & Kiltie 2007).

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Most recently it was suggested that background colours of some eggshells evolved to signal intraspecifically the phenotypic quality and/or condition of the laying female (or the offspring) to promote higher contributions from the male partner through courtship feeding, nest defence, maintenance of nest hygiene and brood provisioning effort (Moreno & Osorno 2003). Observational, correlative and experimental tests of this hypothesis have resulted in mixed results regarding both the underlying assumptions and the predicted outcomes of manipulating blue–green egg coloration (reviewed in Reynolds *et al.* 2009). The majority of research examining the sexually selected eggshell coloration (SSEC) hypothesis has focused on species in the superfamily Muscicapidae (flycatchers and large-bodied thrushes). The Muscicapidae is an important group for study of egg colour, as they are well known for their diversity of eggshell appearance (Lack 1958, Kilner 2006) (Fig. 1) and their varied nest architecture. In addition, most of the species are rarely parasitized by interspecific brood parasites and show low rates of rejection of conspecific or mimetic model eggs (e.g. Grim & Honza 2001, Moskát *et al.* 2003, Boulton & Cassey 2006, Siefferman 2006, Hale & Briskie 2007).

Moreno *et al.* (2004) noted that a premise of the SSEC hypothesis is that eggshell coloration is significantly repeatable within clutches. A further fundamental assumption of intraspecific signalling and communication hypotheses is that the receiver is capable of discriminating the colours of eggs between different clutches. Human estimates or physical reflectance measurements of eggshell coloration do not account for what is processed and discriminated by the avian receivers' (e.g. conspecific mates) eyes and sensory systems (Endler & Mielke 2005). Thus, to understand fully the mechanisms driving the evolution of egg appearance, it is critical to assess how physical (reflectance-based) indices of coloration relate to the limits of avian perception (Bennett & Théry 2007).

In the following analyses, we used a well-established visual model (Vorobyev & Osorio 1998, Vorobyev *et al.* 2001), which estimates photoreceptor noise in the eye, to infer visual thresholds for perceiving colour differences. Perceptual discriminability was estimated (pairwise) between reflectance measures from different eggs within a clutch and between clutches (within a species). We predicted that for the selection of intraspecific avian-visible egg colour polymorphism to be evolu-

tionarily relevant, eggs from different clutches should be easily discriminable from those in other clutches of the same taxa, and more discriminable than eggs within the same clutch, in terms of their colour. This prediction was motivated by the SSEC hypothesis, but is also applicable to all other intraspecific signalling and parasitism hypotheses. Finally, we calculated the commonly applied physical metric of blue eggshell colour (i.e. blue–green chroma; following Siefferman *et al.* 2006), and tested whether average eggshell colour of a blue-type species was related to median inter-clutch discriminability.

METHODS

Materials and data sources

Clutches from 46 species of 44 genera in the superfamily Muscicapidae were measured at the Natural History Museum, Tring, UK. Although the nests and eggs of about one-third to one-quarter of the world's bird species may still be undiscovered or undescribed, the Natural History Museum egg collection is believed to be the most comprehensive in the world, with an estimated 1 million eggs (Knox & Walters 1992). Depending on the samples available in this collection, between three and five clutches of each species were measured, with up to five eggs from each clutch. Thus, the total number of eggs measured was 653, varying from five to 25 per species, with an average of three clutches and over 13 eggs per species.

Despite the large number of species sampled, two possible shortcomings with basing our results solely on the museum samples are: (i) that the majority of these samples were collected almost a century ago (average year = 1913, range = 1856–1972), and (ii) that the average number of eggs, from likely independent clutches, per species is insufficiently large for robust conclusions to be drawn. Thus, we also analysed perceptual model data from much larger samples of Common Blackbird *Turdus merula* ($n = 78$ clutches) and Song Thrush *T. philomelos* ($n = 102$ clutches) eggs collected in the field in New Zealand (data described in Cassey *et al.* 2008a). Both species are exotic to New Zealand and widely persecuted as horticultural pests. All sampling was conducted on private land with the express permission of the landowners and under licence (no. 0443) from the New Zealand Department of Conservation.

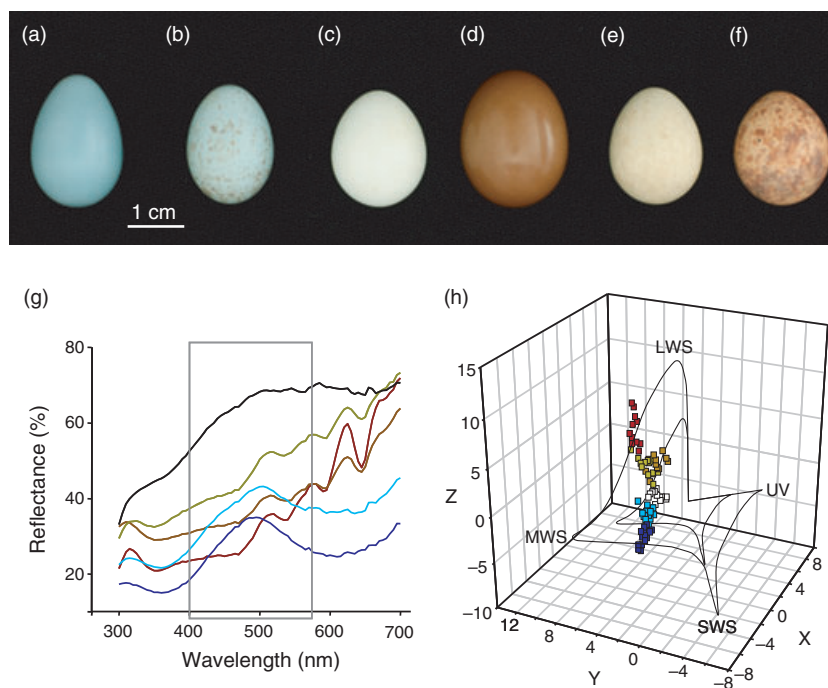


Figure 1. Eggs from six different Muscicapoidae species: (a) White-bellied Redstart *Hodgsonius phaenicuroides* (dark blue line in g), (b) Familiar Chat *Cercomela familiaris* (cyan line), (c) Sooty Chat *Myrmecocichla nigra* (black line), (d) Chorister Robin-chat *Cossypha dichroa* (red-brown line), (e) Black-backed Forktail *Enicurus immaculatus* (brown line), (f) Northern Black Flycatcher *Melaenornis edolioides* (olive-green line). The average reflectance spectra (g) for each of the six eggs (line colours are given in a–f). The grey box shows the proportion of the reflectance wavelength ($R_{400-575}$) used to calculate the blue–green chroma index of Siefferman *et al.* (2006). The tetrachromatic diagram (h) of all sampled eggshell colours for the species in a–f (loci colours are the same as the line colours in g). Derivation of the loci and contours follows Appendix B in Kelber *et al.* (2003). UV, ultraviolet sensitive; SWS, short-wavelength sensitive; MWS, medium-wavelength sensitive; LWS, long-wavelength sensitive.

Physical reflectance of light from eggshells (Cherry & Bennett 2001) was measured using an Ocean Optics USB2000 Miniature Fiber Optic Spectrometer with illumination by a DT mini-lamp. A custom-built light-proof cap was fitted over the probe to maintain a consistent angle (90°) between the eggshell and the measuring fibre optics. Spectra were recorded in ~ 0.4 -nm steps and were expressed relative to a white Ocean Optics WS-1 diffuse reflectance standard. To the best of our ability, six measurements were taken from background shell colour avoiding patterned/maculated regions: two in each hemisphere of the eggshell and two at the equator. To minimize measurement error, dark and white standard reflectance calibration measures were taken regularly during sampling. All further analyses were based on an average reflectance spectrum for each egg (see Fig. 1g).

Following Kilner (2006), we classified the background colour of the shell into three human-

perceived categories: white (or cream), brown (ranging from buff to red to dark chocolate brown) and blue (including shades of grey or green or violet) (data kindly provided by R.M. Kilner). We also calculated reflectance-based eggshell colour using a common index of blue–green chroma (following Siefferman *et al.* 2006) as the proportion of total reflectance in the wavelength region ($R_{400-575}$) across the total spectrum ($R_{400-575}/R_{300-700}$).

Visual modelling

To analyse avian perception and discriminability of eggshell colour as potential signals, we used a colour opponency model that calculates the photon catch of each of the four single cone types (used for colour vision) in the avian retina. These photon catch data were used to predict the ability of a bird to distinguish between different eggshell colours. This model has been demonstrated to describe

visual threshold discrimination in birds accurately (Vorobyev & Osorio 1998, Goldsmith & Butler 2003), including behavioural discrimination of egg rejection (Avilés 2008, Cassey *et al.* 2008b).

Specifically, avian spectral sensitivities were obtained from the literature for both a muscicapid species (Common Blackbird, Hart *et al.* 2000) and a species from a non-muscicapid, sister lineage (Common Starling *Sturnus vulgaris*, Hart *et al.* 1998), using analytical expressions for the spectra of visual pigments and oil droplets (Govardovskii *et al.* 2000, Hart & Vorobyev 2005). Given that many early studies exclusively used human perceptions of egg colour (reviewed in Underwood & Sealy 2002, Kilner 2006), we also contrasted the predicted ability of birds and humans to discriminate eggshell colour signals by constructing a human spectral sensitivity model (see also Cheney & Marshall 2009) using the fundamentals given by Smith and Pokorny (1975).

Because perceived eggshell colours depend upon the interaction between ambient light colour and the reflectance spectra of the eggshells, we compared (from the literature) the effect of six different ambient light spectra (irradiances). These light spectra include the illuminant 'D65', which mimics standard daylight (Wyszecki & Stiles 1982), temperate forest shade 'green' light (Chiao *et al.* 2000), 'grey' light blocked by clouds (Endler 1993), light from small forest 'gaps' (Endler 1993), woodland 'canopy-filtered green' light (Endler 1993) and cavity light from a hole-nesting bird (Jourdie *et al.* 2004). We assumed that together these six irradiances mimic the light conditions expected under natural conditions for most of the species in our dataset, but we recognize that future comparative data are clearly required to make comparisons of environmental light content and nest reflectance properties (see also Avilés *et al.* 2006).

For an average eggshell reflectance spectrum, we calculated the quantum catch q_i of each of the four single photoreceptor cone types (UV-wavelength

sensitive, short-wavelength sensitive, medium-wavelength sensitive, long-wavelength sensitive; designated by $i_{[1-4]}$) over the visible spectrum (300–700 nm in birds), as the integrated product of the spectral sensitivity of cone type i (R_i), the reflectance spectrum of an eggshell (S), and the ambient light spectrum illuminating the nest environment (I).

$$q_i = k_i \int_{\lambda} R_i(\lambda)S(\lambda)I(\lambda)d\lambda. \quad (1)$$

In Equation 1, k is the von Kries adaptation coefficient (Vorobyev *et al.* 1998, 2001), which accounts for the adaptation of the quantum catch to its background. For the log-linear version of the receptor noise model, the stimulation output f is the natural logarithm of q , for each cone type i :

$$f_i = \ln(q_i). \quad (2)$$

The pairwise contrast Δf between average spectra (from different eggs) for each cone type i is then given by:

$$\Delta f_i = f_i(\text{spectra1}) - f_i(\text{spectra2}). \quad (3)$$

Perceptual differences, Δ_e , between two spectra depend on both the difference in cone signals, Δf_i , and the magnitude of the noise-to-signal ratio of cone mechanisms (Weber fraction), ω_i . Values for ω_i were estimated from the literature for birds ($\omega_1 = 0.1$, $\omega_2 = 0.07$, $\omega_3 = 0.07$, $\omega_4 = 0.05$; Vorobyev *et al.* 2001) and humans ($\omega_1 = 0.087$, $\omega_2 = 0.019$, $\omega_3 = 0.017$; Wyszecki & Stiles 1982).

In the case of trichromatic vision (human model) perceptual differences can be expressed in just noticeable differences (jnd) as:

$$\Delta_e = \sqrt{\frac{[e_1^2(\Delta f_3 - \Delta f_2)^2 + e_2^2(\Delta f_3 - \Delta f_1)^2 + e_3^2(\Delta f_2 - \Delta f_1)^2]}{[(e_1e_2)^2 + (e_1e_3)^2 + (e_2e_3)^2]}}.$$

In the case of tetrachromatic vision (avian model) perceptual differences can be expressed as:

$$\Delta_e = \sqrt{\frac{[(e_1e_2)^2(\Delta f_4 - \Delta f_3)^2 + (e_1e_3)^2(\Delta f_4 - \Delta f_2)^2 + (e_2e_3)^2(\Delta f_4 - \Delta f_1)^2 + (e_1e_4)^2(\Delta f_3 - \Delta f_2)^2 + (e_2e_4)^2(\Delta f_3 - \Delta f_1)^2 + (e_3e_4)^2(\Delta f_2 - \Delta f_1)^2]}{[(e_1e_2e_3)^2 + (e_1e_2e_4)^2 + (e_1e_3e_4)^2 + (e_2e_3e_4)^2]}}. \quad (4)$$

It is important to note that the actual receptor noise values differ among individual animals, including humans (Wyszecki & Stiles 1982). To account for such variation, we follow previous empirical studies on the discrimination of spectra by birds and assume that the spectra with Δ_e values < 1 jnd are impossible to discriminate and that those with values < 3 jnd are difficult to distinguish even under reasonable viewing conditions (see discussion in Vorobyev *et al.* 1998, Siddiqi *et al.* 2004, Endler & Mielke 2005).

All statistical analyses were conducted in SAS v 8.02. Visual models were coded in the SAS Interactive Matrix Language following the equations given in Kelber *et al.* (2003).

RESULTS

Across species, the modelled ability to discriminate inter-clutch eggshell colour was similar for both avian visual models, although the Starling discriminated slightly better than the Blackbird (average difference of 4.3% at 1 jnd) (Fig. 2). Unsurprisingly, both avian models discriminated substantially better than the human model (average difference of 21.4% at 1 jnd) (Fig. 2). For each of the models it

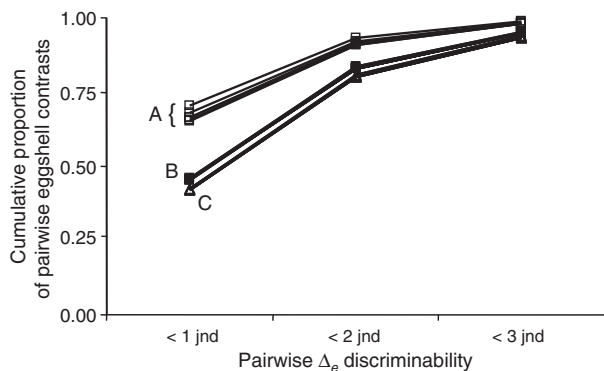


Figure 2. The cumulative proportion of pairwise eggshell contrasts with increasing discriminability (Δ_e) in just noticeable differences (jnd) for chromatic signals as discerned by (A) *Homo sapiens*, (B) *Turdus merula*, and (C) *Sturnus vulgaris*. For each set of contrasts there are six overlapping lines for each of the six natural irradiances (see Methods). This figure gives an estimate of the probability that any two eggshell spectra will *not* be discriminable. For example, the higher the proportion of pairwise contrasts with jnd < 1 , the worse the performance. As jnd increases, the cumulative proportion will eventually reach 1.0 when all pairwise contrasts have values less than the maximum on the x-axis. Each set of contrasts was calculated between eggs of different clutches (but within the same species) for all 46 Muscicapoidae species in six different light environments (irradiances).

was statistically impossible to distinguish between the results from different light environments (irradiances) (Fig. 2). Thus, for brevity, the remainder of the results are calculated using the visual model for the Muscicapid species (*T. merula*) and woodland irradiance (canopy-filtered green light).

Across the sampled taxa, comparisons of perceived egg colours showed that intra-clutch discriminability was lower than inter-clutch discriminability (Fig. 3). The difference between inter-clutch median discriminability and intra-clutch median discriminability was highly significant across species (paired *t*-test between medians, $n = 46$ species, $t = 4.29$, $P < 0.001$). The field samples for *T. merula* and *T. philomelos* also provided results congruent with those from the museum specimens for both median intra-clutch median discriminability (*T. merula* = 0.87, *T. philomelos* = 0.62) and inter-clutch median discriminability (*T. merula* = 1.20, *T. philomelos* = 1.09) (Fig. 3).

Closer examination of the data revealed that the majority of species (43 of 46) had median pairwise Δ_e contrasts of eggshell colour between different clutches < 2 jnd, i.e. differences in colours that are predicted to be difficult to discriminate even under reasonable viewing conditions (Fig. 3b). Furthermore, 28% of species (13 of 46) had median eggshell contrasts between different clutches < 1 jnd, i.e. differences in colours that are predicted to be indistinguishable (Fig. 3b). For both intra-clutch and inter-clutch discriminability there was no significant difference in median pairwise Δ_e between the three eggshell colour categories (Fig. 3; intra-clutch $F_{2,43} = 0.15$, $P = 0.859$ and inter-clutch $F_{2,43} = 0.21$, $P = 0.810$).

Half of the species sampled (23 of 46) had a background eggshell colour previously categorized to be human-perceived 'blue' (following Kilner 2006). In turn, a physical measure of the average blue structural colour (i.e. blue-green chroma) was significantly negatively related to the median pairwise discriminability (between clutches), across species within the 'blue' eggshell colour category (slope \pm se = -9.55 ± 2.50 , $R^2 = 0.41$, $n = 23$, $t = -3.82$, $P = 0.001$).

DISCUSSION

A central aim of our analysis was to demonstrate how researchers testing adaptive explanations for eggshell colour variation can consider the visual system of the relevant signal receivers, be they

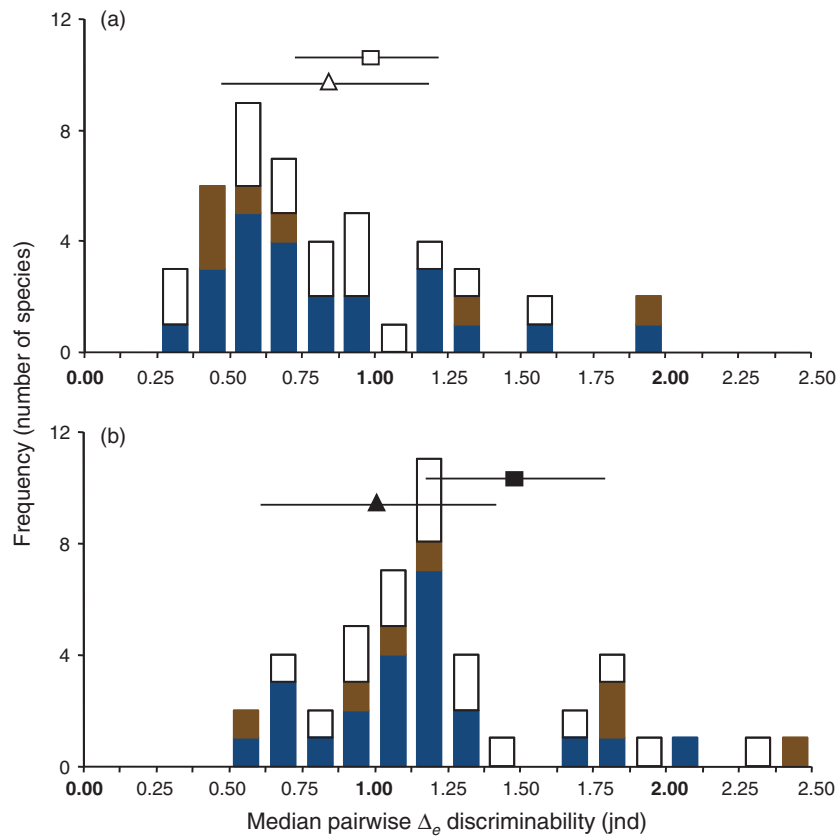


Figure 3. Frequency histograms of median just noticeable differences (jnd) for 46 Muscicapoidae species calculated between eggs within a clutch (a) and between eggs from different clutches (b). The species are classified according to their three different human-perceived background eggshell colours (blue, brown, white; as discussed in the text). Also included are average jnd (\pm se) for clutches of fresh eggs of *Turdus merula* (square symbol) and *Turdus philomelos* (triangle symbol) collected in horticultural fields in New Zealand (data from Cassey *et al.* 2008a).

predators, brood parasites, competitors or the breeding individuals themselves. Here we have used museum samples because they offer a breadth of taxonomic sampling unavailable from field studies. We recognize that because these eggs were collected between 1856 and 1972 (average 1913), their physical and chemical characteristics may have changed due to ageing (Starling *et al.* 2006, Avilés *et al.* 2007). Nevertheless, in Figure 3 we show that for at least two Muscicapid species average pairwise discriminability in a large number of recently studied clutches in exotic populations of these taxa (from Cassey *et al.* 2008a) is of similar magnitude to our museum data. It remains to be shown how labile eggshell properties are with respect to large temporal sequences, and among different species and pigment types (Kennedy & Vevers 1976).

Our suggestion is that under current models of avian perception almost half of all eggs sampled (a minimum of 45%; Fig. 3), and 'blue'-type species (a minimum of 43%; Fig. 3b), are not discriminable from those in other clutches of the same species in terms of their background colour. Indeed, the more blue the eggs the less discriminability. This finding is a fundamental motivation for future investigation to test experimentally behavioural predictions of any signalling- or communication-based hypothesis that looks to explain the evolution of avian-visible egg colour polymorphism through selection at the intraspecific level. In particular, discrepancy between perceptual modelling and behavioural tests will be critical in addressing the role of interspecific variability in other cues used for egg discrimination in birds, including egg-shape, egg-size and the socio-ecological context

of the reproductive cycle (Langmore *et al.* 2003, Grim 2005, Moskát & Hauber 2007). It is also highly likely that discrimination is enhanced when simultaneous comparison of eggs is possible (Gaston *et al.* 1993, Lyon 2003, Honza & Moskát 2005, Moskát & Hauber 2007).

The SSEC hypothesis (Moreno & Osorno 2003) has revitalized research into the mechanisms, perception and adaptive benefits of avian eggshell pigmentation outside the applied field of poultry eggshell research (Reynolds *et al.* 2009). To date, however, evidence from experiments and observations to support the SSEC hypothesis is mixed. Moreno *et al.* (2005) concluded that (when correcting for female age) higher female immunocompetence was associated with darker eggs in Eurasian Pied Flycatchers *Ficedula hypoleuca* (i.e. a luminance trait of eggshell appearance). Thus in addition to spectral differences, signal receivers may also use differences in luminance contrast to distinguish eggshell signals. In birds, double-cone receptors are thought to be responsible for luminance discrimination, as opposed to colour (for discussion see Goldsmith & Butler 2005). In our models, inter-clutch eggshell contrasts from the avian double-cone were consistently less discriminable than chromatic contrasts (results not shown). However, these results suggest that our predictions are not prematurely excluding the ability of birds to distinguish between eggs by over-emphasizing the lack of discriminability between hues (colour) rather than intensity (luminance).

As a result of our sampling protocol, we analysed eggs of species from a single avian superfamily but whose eggs show a wide range of colours, from (as they appear to humans) pure white through chocolate brown to blue (e.g. Fig. 1a–f). The SSEC hypothesis was originally proposed to explain variation in the base colour of eggshells with a particular focus on blue-green coloration from the bile pigment biliverdin (Moreno & Osorno 2003). It is interesting to note that more recently, two studies (Martínez-de la Puente *et al.* 2007, Sanz & García-Navas 2009) have also proposed broadening the applicability of the SSEC hypothesis to red-brown (protoporphyrin)-based maculation of eggshell patterning. The presence of porphyrins and bile pigments in eggshells was recognized nearly a century ago (Fischer & Kögl 1923, Lemberg 1934), and most recently characterized by HPLC/electrospray ionization tandem mass spectrometry (Gorchein *et al.* 2009). These tetrapyrrole pigments are

involved in the synthesis and catabolism of haem (Burley & Vadhera 1989, Milgrom 1997, Bauer & Bauer 2002) and are both circulating in the bloodstream and metabolized *de novo* in the shell gland (Poole 1966, Solomon 1997). Moreno and Osorno (2003) proposed that the intensity of biliverdin-based coloration might signal the female's antioxidant capacity, and there is recent evidence that eggshell colour may indeed be costly in terms of circulating antioxidants (Morales *et al.* 2008). Biliverdin has been detected in eggshells of all three categorical colour types (Kennedy & Vevers 1976), and although the production of eggshell pigments is suggested to be under independent genetic control (Collias 1993), both may be produced simultaneously (but in different concentrations) to produce the variety of perceived differences in appearance. Whereas birds excrete biliverdin directly, most of the work on non-avian taxa has focused on the antioxidant role of its reduced metabolite bilirubin (Stocker *et al.* 1987). We suggest that studies confirming the specific antioxidant roles (and costs) of biliverdin and other common eggshell pigments will be greatly rewarding.

The overall poor predicted ability of birds in our taxonomic sample to discriminate between different clutches (across a wide range of eggshell colours) clearly demonstrates that accounting for the ability of a bird's eye markedly alters the evolutionary interpretation of eggshell coloration. This conclusion was robust to both a range of different irradiance spectra (nest light environments) and a range of spectral sensitivities of two passeriform bird species which forage (and nest) in a variety of different terrestrial habitats (Hart 2001). In addition, median pairwise discriminability was not related to a categorical classification of background eggshell colour (white, brown or blue). For blue-type eggs, median pairwise discriminability was strongly negatively related to a physical measure of the variability in structural colour (blue-green chroma), suggesting that pairwise discriminability (between clutches) is lowest in species with the highest relative reflectance of blue wavelengths.

We know very little about the physiological costs of eggshell coloration or the specific chemical differences that underlie perceivable variability in eggshell colour (Kennedy & Vevers 1976). Our results call for further research into whether eggshell colour, in general, is an intraspecifically detectable communication signal or recognition cue, and we would particularly welcome further studies that

provide a wider range of species-specific data to test our findings. Importantly, we propose that understanding the evolutionary significance of variability in avian eggshell appearance will require future studies that test alternative signalling and structural hypotheses in concert rather than in isolation (e.g. Magie *et al.* 2008).

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