



**Avian color perception predicts behavioral responses to experimental brood parasitism in chaffinches**

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2 **Avian color perception predicts behavioral responses to experimental brood**  
3 **parasitism in chaffinches**

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19 Online enhancements: table A1, Figures A1 and A2.

**1 Abstract**

2 Hosts of cuckoos have evolved defenses allowing them to discriminate and reject parasite eggs.

3 Mechanisms of discrimination are mostly visually mediated, and have been studied using

4 approaches that do not account for what the receiver (i.e. host) actually can discriminate. Here, for

5 the first time we apply a perceptual model of color discrimination to study behavioral responses

6 to natural variation in parasite egg appearance in chaffinches *Fringilla coelebs*. Discrimination of

7 parasite eggs gradually increased with increasing differences in chromatic contrasts as perceived

8 by birds between parasite and host eggs. These results confirm that color differences of the eggs

9 as perceived by birds are important integral parts of a matching signal used by chaffinch hosts.

10 *Keywords:* avian vision, cuckoo parasitism, chaffinch, egg discrimination, host perception,

11 sensory visual discrimination

## 1 **Introduction**

2 Cuckoo *Cuculus canorus* – host interactions provide some of the clearer text-book examples of  
3 coevolution in vertebrates. Cuckoos lay their eggs in the nests of host species, and leave parental  
4 care of their offspring to unrelated foster parents (Davies, 2000; Payne, 2005; Stokke *et al.*,  
5 2005). Cuckoo parasitism is harmful for hosts since once the cuckoo egg hatches the young  
6 cuckoo readily displaces all host eggs and chicks (Davies, 2000), which causes a dramatic  
7 reduction of host reproductive output (Øien *et al.*, 1998; Krüger, 2007). Cuckoo parasitism has  
8 therefore selected for effective defensive mechanisms in their hosts, which at the same time has  
9 selected for further elaborated counter-defenses in the cuckoo to overcome host defenses (Brooke  
10 & Davies, 1988; Davies & Brooke, 1988; Stokke *et al.*, 2005). In this coevolutionary arms race  
11 scenario, many host species have evolved anti-parasite defenses allowing them to defend  
12 themselves against parasite exploitation by discriminating and rejecting any odd-looking egg that  
13 is added to their clutches (Rothstein, 1990; Moksnes *et al.*, 1991).

14 Evidence suggests that stimuli involved in cuckoo egg discrimination are mostly visually  
15 mediated (Davies, 2000). A number of studies have shown that cuckoo egg rejection increases  
16 with differences in appearance between cuckoo and host eggs as judged by humans (Davies,  
17 2000). Many bird species, including cuckoo hosts, can perceive UV wavelengths, of which  
18 humans are blind, due to a fourth cone type in their retinas which is sensitive to UV light (e.g.,  
19 Bennett *et al.*, 1996; Bowmaker *et al.*, 1997; Cuthill *et al.*, 2000). Recent application of  
20 spectrophotometry that accounts for UV wavelength has revealed the possible existence of  
21 cuckoo genes that appear to be cryptic to human vision (e.g., Cherry & Bennett, 2001; Avilés &  
22 Møller, 2004; Starling *et al.*, 2006). However, a limitation in these studies is that egg-matching is  
23 quantified as the difference in reflectance between cuckoo and host eggs. Differences in

1 reflectance may include spectral information which is not perceived by hosts (Cuthill *et al.*,  
2 2000). Also, that approach does not account for what the host eyes actually perceive, and  
3 disregard the luminal environment where the eggs should be discriminated (Vorobyev *et al.*,  
4 1998; Endler *et al.*, 2005). Thus, although differences in reflectance have proved to successfully  
5 predict rejection of parasitic eggs, these associations would more likely be detected when  
6 considering actual host visual perception. Avilés (2008) recently used realistic models of hosts'  
7 perceptual physiology that also account for nest luminosity to study perception of different  
8 cuckoo egg morphs in relation to host vision. However, discrimination experiments are clearly  
9 needed to quantify the evolutionary selective pressures acting on cuckoo egg coloration  
10 considering what is known about hosts' visual abilities and the light conditions of host nests.

11 In a recent work, Cassey *et al.* (2008) found that differences in quantum catches from the  
12 ultraviolet and short-wavelength sensitive cones predicted discrimination of artificial eggs in song  
13 thrushes *Turdus philomelos*. Although they followed a visual model approach to calculate  
14 differences in quantum catches between host and artificial eggs at each cone, they failed to report  
15 a whole effect of chromatic distance on egg discrimination. Furthermore, the colors used to paint  
16 parasite eggs in that study did not reflect ultraviolet wavelengths and displayed unnatural shades  
17 at longer wavelengths (Honza *et al.* 2007), which resulted in an unnaturally high discriminability  
18 of experimentally painted eggs (Cassey *et al.*, 2008). Both intra-specific brood parasitism and  
19 cuckoo parasitism with mimetic eggs are likely to challenge hosts with difficult discriminatory  
20 tasks, and it is therefore critical to establish the limits of host sensory perception by studying host  
21 behavioral responses to natural variation in egg coloration. Furthermore, egg discrimination by  
22 hosts may rely on differences between parasite and host eggs in other characteristics than  
23 coloration (Stokke *et al.*, 2007), such as pattern of spottiness (e.g., Lahti & Lahti, 2002), shape

1 (e.g., Underwood & Sealy, 2006) or size (e.g., Marchetti, 2000) that can not easily be controlled  
2 for in a visual model approach. This may be particularly problematic if host eggs show complex  
3 patterns of egg-shell maculation or if parasite and host eggs are very different in size and/or  
4 shape.

5         Here we used a visual model approach to assess the relative importance of host color  
6 discrimination for the task of parasite egg rejection in chaffinches *Fringilla coelebs*. Chaffinches  
7 are known to efficiently reject model eggs at high rates although they are not currently being  
8 parasitized by cuckoos (Braa *et al.*, 1992; Moksnes, 1992; Stokke *et al.*, 2004). The existence of  
9 chaffinch clutches parasitized with cuckoo eggs that perfectly mimic chaffinch eggs (*Fringilla*  
10 cuckoo egg type *sensu* Moksnes & Røskaft, 1995; Avilés & Møller, 2004) in egg collections  
11 dating one century back suggests that chaffinches were actively exploited by cuckoos in the recent  
12 past, and that egg rejection behavior in chaffinches has evolved in response to cuckoo parasitism  
13 (Stokke *et al.*, 2004). The main prediction emerging from sensory discrimination theory in the  
14 coevolutionary arms race scenario between the cuckoo and its hosts was that (i) the likelihood of  
15 rejecting foreign eggs should decrease as the stimulus perceived by hosts become more similar.

16

## 17 **Methods**

### 18 **Study area and experimental procedure**

19 The study was carried out in Stjørdal, approx. 30 km east of Trondheim in central Norway  
20 (63°10'N, 10°20'E) during April-May 2007-2008. The study area consists of three minor lowland  
21 grey alder *Alnus incana* woodlands in which chaffinches breed at high densities and cuckoos are  
22 currently absent (Stokke *et al.*, 2004).

1           A total of 74 successful experiments are included in this study. In order to simulate  
2 cuckoo parasitism behavior, we replaced one randomly selected chaffinch egg in each clutch with  
3 a conspecific egg from another nest on the day the fourth or fifth egg was laid. This procedure  
4 allowed the hosts to assess the appearance of their whole clutch before taking a rejection decision  
5 (see Stokke *et al.*, 2004). The removed egg was then used in another experiment. Host and  
6 parasite eggs were photographed in the field on a neutral grey plate together with a ruler and a  
7 color reference using a Canon EOS 30D camera and a 100mm macro lens (f1/2.8). Photographs  
8 provide an easy way to assess patterns of spottiness, shape and volume (see below), although they  
9 do not capture the variation in coloration in the entire bird-visible waveband. There are  
10 remarkable differences in egg appearance among different chaffinch females in our population  
11 (fig.1A,B; Stokke *et al.*, 2004). Therefore, our egg-exchange experiment produces a smooth  
12 gradient of color matching between parasite and host eggs from the host's perspective. The host's  
13 response was recorded as either rejection (parasite egg ejected) or acceptance if the parasite egg  
14 had been incubated for at least five days.

15

## 1 **Spectral measurements of eggs and nest luminosity**

2 We estimated coloration (i.e., spectral reflectance at the 300-700 nm waveband) of host and all  
3 experimentally exchanged chaffinch eggs with an Ocean Optics spectrometer equipment [S2000  
4 spectrometer connected to a deuterium-halogen light (D2-W, mini) by a coaxial reflectance probe  
5 (QR-400-7-UV-vis) and the OOIBase32<sup>TM</sup> operating software (Ocean Optics, Inc. Dunedin, FL,  
6 USA)]. A stratified random sample of spectra from all regions of the eggs was obtained by  
7 dividing each egg in three bands around the long axis (e.g., Cherry & Bennett, 2001; Avilés *et al.*,  
8 2004; 2006a, 2006b; Starling *et al.*, 2006). Color was measured in each of these three bands  
9 (twice in the central band and once in each of the distal bands) deliberately avoiding scans within  
10 spots. Chaffinch eggs are not densely spotted and huge spot-free portions of the background are  
11 always predominant over spotted egg-shell areas (see fig 1A,B). Therefore, for this particular  
12 species, and, given low spot density and size, our approach reliably captures most of the variance  
13 in egg color. Reflectance was measured with the probe placed at a constant distance and at a 45°  
14 angle. Measurements were relative to a standard white (WS-2) and to the dark, which was  
15 calibrated before the measurement of each clutch. All the measurements were performed in a dark  
16 room indoors to avoid an effect of ambient light on spectro-measurements. We estimated average  
17 coloration of chaffinch eggs in a clutch by sampling one single host egg per clutch, which is  
18 justified by an extraordinary high consistency in coloration among eggs within a clutch in this  
19 species (fig.1A,B; Stokke *et al.*, 2002; 2004). Unless a nest was deserted, we did not measure  
20 more than one egg from each clutch, and that egg was used as the experimental parasitic egg in a  
21 subsequent experiment. Therefore, the four measurements from each egg were averaged to give a  
22 mean host spectrum for each clutch. The average spectral distribution of sampled chaffinch eggs  
23 is shown in fig. 1C.

1 Ambient light measurements were collected in the morning between 10.00 h and 12.00 h  
 2 on May 16<sup>th</sup> and 17<sup>th</sup> 2008 at six deserted nests which each were mounted in five randomly  
 3 selected nest sites in our study area. To get representative irradiance we collected two different  
 4 sets of measurements in one hour at every nest. We do not have information on the time of day  
 5 when chaffinches recognize the eggs, but video recordings have shown that a significant  
 6 proportion of parasite eggs are rejected in the morning among other cuckoo hosts (Martín-Vivaldi  
 7 M., Com. Pers.). Briefly, we used a cosine-corrected fiber-optic probe (P400-2-UV-VIS; Ocean  
 8 Optics) with a 180° angle of acceptance and a measurement surface of 6 mm in diameter (CC-3-  
 9 UV; Ocean Optics). The spectrometer was calibrated with a light source of known color  
 10 temperature (LS-1-CAL; Ocean Optics). We measured the ambient light (three readings per nest)  
 11 close to the nest floor (i.e. where parasite and host eggs were placed) with the measurement  
 12 surface oriented to the sky, and the probe held perpendicular to the ground. We transformed  
 13 irradiance readings into photon units, as described by Endler (1990), and calculated the average  
 14 irradiance spectrum in chaffinch nests in our population for model calculations (fig. 1D).

15  
 16 Fig. 1 about here

### 17 **Avian color space modeling**

18 *Chromatic Contrasts.* We calculated discriminability of parasite eggs for each experiment using  
 19 the model of Vorobyev & Osorio (1998) developed for the tetrachromatic visual system of birds  
 20 in its log form (Vorobyev *et al.*, 1998). This model establishes a color distance  $\Delta S$  which  
 21 describes the color contrasts between two eggs as:

$$\begin{aligned}
 \Delta S^2 = & [(e_1 e_2)^2 (\Delta f_4 - \Delta f_3)^2 + (e_1 e_3)^2 (\Delta f_4 - \Delta f_2)^2 + (e_1 e_4)^2 (\Delta f_2 - \Delta f_3)^2 \\
 & + (e_2 e_3)^2 (\Delta f_4 - \Delta f_1)^2 + (e_2 e_4)^2 (\Delta f_3 - \Delta f_1)^2 + (e_3 e_4)^2 (\Delta f_2 - \Delta f_1)^2] / \\
 & [(e_1 e_2 e_3)^2 + (e_1 e_2 e_4)^2 + (e_1 e_3 e_4)^2 + (e_2 e_3 e_4)^2]
 \end{aligned}$$

(1)

1  
2 where  $\Delta f_i$  is the log ratio of the quantum catches of each class of single cones (long-wavelength  
3 sensitive [LWS], medium-wavelength sensitive [MWS], short-wavelength sensitive [SWS],  
4 ultraviolet wavelength sensitive [UVS]) denoted by the subscript for cone  $i$ , for host (H) and  
6 parasitic (P) eggs.

$$\Delta f_i = \log \frac{\int_{300}^{700} R_H(\lambda) I(\lambda) S(\lambda) d\lambda}{\int_{300}^{700} R_P(\lambda) I(\lambda) S(\lambda) d\lambda}$$

(2)

18  
19 where  $R_H(\lambda)$  represents the average reflectance of the host eggs,  $R_P(\lambda)$  is the average reflectance of  
20 the parasite egg in a given chaffinch clutch,  $I(\lambda)$  is the spectral irradiance at the nest, and  $S(\lambda)$  is  
21 the spectral sensitivity of host  $i$ . Results of calculations using equation (1) provide the chromatic  
22 distance  $\Delta S$  separating the perceptual value of two eggs in host receptor space. The units for  $\Delta S$   
23 are JNDs (just noticeable differences). Essentially, colors that appear similar to a signal receiver  
24 (either because of the nature of their visual system or an absolutely small difference in the  
25 reflectance spectra of the colors) result in small  $\Delta S$  values, while those that have high chromatic  
26 contrast have large  $\Delta S$  values (Osorio & Vorobyev, 1996). Spectral sensitivity has not been  
27 measured in the chaffinch. Therefore, following recently published literature (e.g., Håstad *et al.*,  
28 2005; Gómez & Théry, 2007; Avilés *et al.* 2008; Avilés, 2008; Håstad & Ödeen, 2008) we used  
29 spectral sensitivity data from the blue tit *Cyanistes caeruleus* with SWS1 : SWS2 : MWS : LWS  
30 cone proportions of 1: 1.92: 2.68: 2.7 (Hart *et al.*, 2000) as representative of a UVS system, and  
31 assumed that the signaling noise  $e_i$  for each cone was independent of light intensity.

$$e_i = \omega / \sqrt{\eta_i}$$

(3)

where  $\omega$  is the Weber fraction (taken as 0.05 (Vorobyev *et al.*, 1998)) and  $\eta_i$  is the relative density of the cone class  $i$  on the retina. The version of the Vorobyev-Osorio model used here assumes daylight conditions, which is justified because parasite egg rejection seems to always occur under daylight conditions (Martín-Vivaldi M., Com. Pers.), and because the chaffinch build open and shallow nests (Cramp, 1998).

Spectral sensitivity is affected by the relative cone proportions in the bird retina, which may vary from one species to another (Hart, 2001). We therefore assessed the sensitivity of our analysis to variation in cone proportions by repeating color contrast calculations for all the six UVS species found by Hart (2001). Estimations of differences in chromatic contrasts between parasite and hosts eggs were strongly correlated ( $r^2 > 0.99$ ,  $P < 0.00001$  for all the 15 possible combinations). Furthermore, the averaged estimate of chromatic contrast based on blue tit cone proportion fell within 1 SD of the means calculated using all known cone proportions for UVS species in Hart (2001) (mean/SD: blue tit: 2.77/1.19; blue-faced honeyeater *Entomyzon cyanotis*: 2.76/1.19; noisy miner *Manorina melanocephala*: 2.63/1.13; satin bowerbird *Ptilonorhynchus violaceus*: 3.02/1.31; european starling *Sturnus vulgaris*: 2.89/1.24; blackbird *Turdus merula*: 2.47/1.05). Thus our results were robust to variations in the relative cone proportions of the bird retina.

*Achromatic Contrasts.* Evidence suggests that birds may use achromatic signals in discriminatory tasks (reviewed in Kelber *et al.*, 2003). In this vein, it has been recently suggested that achromatic mechanisms could play a key role in the discrimination of cuckoo eggs at low-light levels (Avilés,

1 2008). In birds, double cones are assumed to be responsible of achromatic visual detection (e.g.,  
2 Osorio *et al.* 1999a; 1999b). We thus applied equations 1 to 3 to sensitivity data for double cones  
3 of blue tits (Hart *et al.*, 2000). We used the Weber fraction as 0.05 (at threshold) for the double  
4 cone in birds and calculated achromatic (brightness) contrasts similar to the chromatic analysis.  
5 For every experimentally parasitized clutch we therefore calculated chromatic and achromatic  
6 contrasts between the averaged host spectrum and the averaged spectrum for the parasite egg.

7

### 8 **Egg volume and shape**

9 The maximum length and breadth of one randomly selected per clutch host and the parasite eggs  
10 were estimated to the nearest 0.1 mm based on the photographs using digital imaging software  
11 (NIS Elements BR 3.0, Nikon, Amstelveen, The Netherlands). To ensure consistency all  
12 measurements were done by one person (JRV). Egg volume index (EV) was calculated from the  
13 length (EL) and breadth (EB) using the formula  $EV=0.5 \times EL \times EB^2$  (Hoyt 1979). In addition, we  
14 used Picman's (1989) simple shape index (shape =  $EL/EB$ ) to determine egg shape. For every  
15 experimentally parasitized clutch we calculated the absolute value of the difference between  
16 volume and shape between the host and the parasite eggs.

17

### 18 **Patterns of spottiness**

19 Based on the photographs, spot measurements of one host and the parasite egg were carried out  
20 using NIS-Elements BR 3.0. Measurements were delimited to a rectangle of the egg surface  
21 covering 90% of the longitudinal axis and the respective width (see figure 1 in appendix). Spots  
22 were marked with the threshold tool (analogous to the magic wand tool in Photoshop) in the  
23 binary editor of NIS, and three different variables were provided by the program: Spot coverage

1 as the total spot area relative to the background in the rectangle; Perimeter/Area ratio (P/A  
2 hereafter) defined as the sum of the perimeter of all spots divided by the sum of the total spotted  
3 area. This measure allows us to differentiate between patterns of a few but large versus many but  
4 small spots having the same spot coverage. Finally, we used Mean circularity as a measure of spot  
5 shape. Circularity is a derived measure which equals  $4 \cdot \pi \cdot \text{Area} / \text{Perimeter}^2$ . Perfect circles have  
6 circularity 1 while non-circular spots have circularity values between 0 and 1. We estimated  
7 repeatability for the three variables in a set of 20 randomly selected eggs sampled in two different  
8 days. Repeatability proved to be very high for the three variables ( $r = 0.94-0.98$ ,  $F_{19,20} > 23.7$ ,  $P <$   
9  $0.00001$ ,  $N = 20$ ). For every experimentally parasitized clutch we calculated the absolute value of  
10 the difference between the three descriptors of spottiness between the host and the parasite eggs.

11

## 12 **Statistical methods**

13 In chaffinches, the female is the sex responsible for rejection of parasite eggs (Moksnes *et al.*,  
14 1994). Previous intensive catching and ringing of breeding chaffinches in this population has  
15 revealed a negligible chance of sampling the same female in two consecutive years (Stokke *et al.*  
16 2004). We therefore counted every experiment as an independent data point in our analyses. Also,  
17 previous studies revealed no age-specific patterns of recognition and laying in chaffinches  
18 (Stokke *et al.*, 2004). Chromatic and achromatic contrasts as well as contrasts in volume, shape  
19 and spottiness between parasitic and host eggs were not significantly different from a normal  
20 distribution (Kolmogorov-Smirnov tests,  $P > 0.05$ ).

21 We used logistic regression models (GENMOD procedure in SAS, SAS Institute, 1996) to  
22 test whether rejection of parasite eggs in chaffinches (i.e., rejection vs acceptance) was explained  
23 by differences in matching (i.e. chromatic and achromatic contrasts, volume, shape, spot

1 coverage, spot P/A ratio and spot circularity) between host and parasite eggs. Year was entered as  
2 a fixed factor to control for annual variation. From this global model, we constructed candidate  
3 models using the main effects of each predictor. According to Green (1979) any correlation less  
4 than 0.70 eliminates serious problems of collinearity in multivariate analyses. None of the  
5 predictors had  $r_p$  values larger than 0.7 (see table 1 in appendix). Due to limited sample size we  
6 discarded those subsets including interactions. The best-fit model was determined using Akaike's  
7 information criterion (Burnham & Anderson 1998). Because the number of datapoints in the  
8 global model divided by  $K$  (the number of parameters in the model) is less than 40, AIC was  
9 corrected for small sample sizes (known as AICc) following Burnham & Anderson (1998). The  
10 model with the lowest value of AICc is the most parsimonious one in the sense that it provides  
11 the best balance between overfitting (hence loss of precision) and underfitting (hence bias) and is  
12 the selected model. The Akaike weights give the relative support for a given model compared to  
13 the other models in the set (all information in Burnham & Anderson 1998). Furthermore, we also  
14 employed model averaging (Burnham & Anderson, 1998; Symonds & Johnson, 2008) to identify  
15 more accurately the relative importance of each model term in predicting rejection of parasite  
16 eggs. This involved taking the subset of the most likely models (cumulative Akaike weight  $\leq$   
17 0.95), adjusting the Akaike weight for each model accordingly, and then calculating Akaike  
18 weights for each model term by summing the Akaike weights for each model in which the term  
19 features. These scores range from 0 (the term appears in none of the most likely models) to 1 (the  
20 term appears in all of the most likely models) (Symonds & Johnson, 2008).

21

## 22 **Results**

### 23 **Egg rejection in relation to parasite-host egg matching**

1 54 out of 74 (72.98 %) experimental parasite eggs were rejected by the chaffinch. There were  
2 eight different models that provided substantial support (i.e.,  $\Delta_i \leq 2$ ) for egg rejection (table 1).  
3 The best model describing the average rejection rate of parasite eggs included chromatic contrast  
4 as the only term, and this term appeared in all models providing substantial support for egg  
5 rejection (table 1). Model averaging showed that chromatic contrast was clearly the most  
6 important predictor of parasite egg discrimination, with an Akaike weight of 0.84, indicating that  
7 it featured in 117 out of the 139 most likely models (table 2). Achromatic contrasts and shape  
8 contrasts were the second and third most important predictors of parasite egg rejection by  
9 chaffinches (Akaike weights of 0.51 and 0.43, respectively, table 2). The remaining predictors of  
10 rejection had low Akaike weights (table 2).

11

12 Table 1 about here

13 Inspection of model average estimates showed that chromatic contrast was the only term for  
14 which the 95% CI did not include zero (table 2). Rejection probability of parasite eggs increased  
15 with chromatic differences between parasite and host eggs (range: from 25.0 % for pairs tested  
16 against parasite eggs differing 0-1 JND to 92.3 % for pairs tested against parasite eggs differing >  
17 4 JND; table 2; fig 2). The model averaged estimates of remaining predictors of rejection had  
18 95% CI's for model averaged estimates that included zero values (table 2).

19

20 Table 2 about here

21 Figure 2 about here

22 **Discussion**

1 To our knowledge, this is the first study in which realistic models of the hosts' perceptual  
2 physiology have been applied to investigate host behavioral responses to natural variation in egg  
3 coloration. The behavioral responses by chaffinches to experimental parasitism using conspecific  
4 eggs partly conformed to expectations from the discrimination model based on host perception of  
5 chromatic differences. The higher the value of chromatic contrasts between the parasite and the  
6 host eggs, the higher was the probability of the parasite egg being rejected. Our findings confirm  
7 the adaptive value of matching as perceived by hosts for a parasite egg, which was previously  
8 known intuitively based on more indirect methods for the assessment of mimicry such as those  
9 based on human vision (e.g., Davies & Brooke, 1988; Moksnes & Røskaft, 1992; Davies, 2000)  
10 and spectrophotometry (e.g., Avilés *et al.*, 2006c; Honza *et al.*, 2007; Polaciková *et al.*, 2007;  
11 Cherry *et al.*, 2007). In addition, our results provide experimental support to previous findings in  
12 other animal taxa (e.g., bees, mice and humans) suggesting that sensory discrimination improves  
13 with the magnitude of the stimulus (e.g., Wolfe, 2000; Abraham *et al.*, 2004; Chittka *et al.*, 2003;  
14 Dyer & Chittka, 2004).

15         Although we found that the probability of egg rejection gradually increased with the  
16 magnitude intensity of the visual stimulus (i.e.,  $\Delta S$ ), chaffinches discriminated between eggs  
17 whose visual stimulus according to the perceptual model would appear similar to a bird receiver  
18 (i.e., small  $\Delta S$  values). In the other direction, chaffinches also accepted a considerable proportion  
19 of clearly contrasting parasite eggs (fig.2). Previous studies have shown that for some bird  
20 species, predicted thresholds for chromatic discrimination are matched by behavioral data on  
21 discrimination of colors against a background under photopic conditions (e.g., Vorobyev &  
22 Osorio, 1998; Kelber *et al.*, 2003; Goldsmith & Butler, 2005). The JND threshold should be  
23 treated as a guideline in this study since we parameterized the model using data from other

1 species. A certain level of mismatching between model predictions and behavioral data has been  
2 reported for dim light conditions, probably due to a higher relative importance of the achromatic  
3 mechanism (Vorobyev & Osorio, 1998). One explanation for the discrepancy between the  
4 predictions of the color discrimination model and the test results in chaffinches is that parasite  
5 egg discrimination may somehow depend on specific aspects of egg appearance (i.e., shape,  
6 volume, spottiness) that are not accounted for by avian chromatic and achromatic contrasts as  
7 measured in this study. Here we have quantitatively assessed differences between parasite and  
8 host eggs in other characteristics than coloration, such as pattern of spottiness, shape or size.  
9 However, it cannot be excluded that our way of quantifying spottiness did not capture how the  
10 host processes the spatial configuration of colors across the egg surface. Nonetheless, our results  
11 confirm that color difference of the eggs is an integral part of the host matching signal. Finally,  
12 although our results were robust to reported cone proportion variations among UVS species (see  
13 methods), spectral sensitivity data for chaffinch were not available in the literature, and therefore  
14 we parameterized the model with blue tit data as representative of typical ultraviolet sensitive  
15 birds.

16 Previous empirical data for humans and other animals have shown that photopic  
17 discrimination is based on predominantly color opponent channels (Vorobyev & Osorio, 1998;  
18 Schaefer *et al.*, 2007). In addition, it has been shown that cuckoo hosts would better perceive  
19 chromatic differences with cuckoo eggs in bright than in dim nest environments (Avilés, 2008).  
20 Chaffinches are typical open nesters and, therefore, discriminatory tasks should be photopic. In  
21 agreement, we have found that chromatic difference as would be perceived by hosts was by far  
22 the best predictor of egg rejection. However, our experiment did not break the correlation  
23 between differences in color and brightness between host and experimental eggs ( $r_p = 0.62$ ,  $P <$

1 0.00001, N = 74 nests). Only future experiments in which the chromatic and achromatic parts of  
2 the compound visual stimulus are manipulated would allow disentangling the role of chromatic  
3 and achromatic signals in parasite egg discrimination.

4 In conclusion, we have documented that difficult discriminatory tasks in terms of visual  
5 discrimination, as predicted by physiological models of host perception, are solved less frequently  
6 by chaffinches than easier discriminatory tasks. Physiological models both under- and  
7 overestimated chaffinch acuity for discriminating parasite eggs. Two previous papers (Cassey *et*  
8 *al.*, 2008; Avilés, 2008) concluded that the use of retinal models was necessary for quantitatively  
9 describing the strength of the selection pressures acting on parasite egg coloration. The findings  
10 of this study highlighted the importance of incorporating realistic models of the host' perceptual  
11 color physiology in conjunction with other candidate components of matching signal (e.g., shape,  
12 volume and spottiness) to the study of egg discrimination by host of avian brood parasites.

13

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1 **Table 1** Most likely models in descending order explaining variation in rejection behavior in chaffinches. The table  
 2 shows the factors included in the model and model estimates (slopes) from logistic regression model that assesses the  
 3 relationship between frequency of experimental egg rejection and the average differences between host and parasite  
 4 eggs in  $\Delta S$ ,  $\Delta Q$ , shape, volume, spot coverage, spot circularity and spot spot P/A ratio. Model selection was based on  
 5 AICc, and  $\Delta AICc$  is the difference between a particular model and the best one within each subanalysis.  $K$  is the  
 6 number of estimated parameters. Akaike weights show the relative support a given model has from the data compared  
 7 with the other models in the set.  $\Delta S$  and  $\Delta Q$  correspond to avian color and achromatic contrasts, respectively.

Model	$\Delta S$ Estimate (s.e.)	$\Delta Q$ Estimate (s.e.)	Shape Estimate (s.e.)	Volume Estimate (s.e.)	Spot coverage Estimate (s.e.)	Spot circularity Estimate (s.e.)	Spot P/A ratio Estimate (s.e.)	Year Estimate (s.e.)	$K$	AICc	$\Delta AICc$	Akaike Weights
Rejection behavior												
$\Delta S$	0.79 (0.27)								3	80.4	0.0	0.051
$\Delta S, \Delta Q$	0.61 (0.32)	0.28 (0.28)							4	81.5	1.1	0.028
$\Delta S, \text{shape}$	0.82 (0.28)		-4.43 (4.33)						4	81.6	1.2	0.028
$\Delta S, \text{year}$	0.83 (0.28)						0.53 (0.57)		4	81.7	1.4	0.025
$\Delta S, \text{volume}$	0.83 (0.28)			-0.001 (0.002)					4	81.9	1.5	0.024
$\Delta S, \text{spot}$	0.82 (0.28)					3.53 (4.59)			4	82.0	1.6	0.024
circularity												
$\Delta S, \text{spot P/A ratio}$	0.82 (0.28)						0.04 (0.06)		4	82.1	1.7	0.022
$\Delta S, \Delta Q, \text{shape}$	0.58 (0.33)	0.37 (0.29)	-5.76 (4.49)						5	82.2	1.8	0.020
$\Delta S, \text{spot coverage}$	0.80 (0.28)				2.05 (7.16)				4	82.5	2.1	0.017



1 **Table 2** Akaike weights for each factor in the most likely models predicting parasite egg  
 2 rejection.  $\Delta S$  and  $\Delta Q$  correspond to avian color and achromatic contrasts, respectively. Estimates  
 3 in bold indicate that 0 is excluded from 95% confidence interval and that variable influences  
 4 rejection of parasite eggs.

5

Term	Akaike Weight (95 %)	Model averaged estimate ( $\pm$ 95 % CI)
$\Delta S$	<b>0.84</b>	<b>0.74</b> <b>(0.13 to 1.35)</b>
$\Delta Q$	0.51	0.47 (-0.08 to 1.02)
shape	0.43	-6.42 (-15.47 to 2.63)
volume	0.36	-0.002 (-0.005 to 0.002)
spot circularity	0.35	5.25 (-4.16 to 14.66)
year	0.28	-0.16 (-0.81 to 0.49)
spot P/A ratio	0.23	0.04 (-0.09 to 0.16)
spot coverage	0.22	2.03 (-12.39 to 16.45)

6 Note: 139 models were identified as most likely (i.e., accounting for a summed  
 7 Akaike weight of 0.95) for parasite egg rejection.

1

2 **LEGEND FOR FIGURES**

3

4 **Fig. 1** Sample photographs of experimentally parasitized chaffinch clutches showing moderate  
5 (A) and high (B) contrast, with the parasite egg on the right in the bottom row. Average  
6 reflectance spectrum (C) of chaffinch eggs and average spectral irradiance at chaffinch nests (D).  
7 Curves are the mean of individual means of four measurements taken at every egg for reflectance  
8 and the mean of individual means of four measurements taken at every nest for irradiance,  
9 respectively. Bars are standard deviations.

10

11 **Fig. 2** Percent of parasite eggs rejected (grey bars) and accepted (white bars) in relation to  
12 chromatic matching estimated under the Vorobyev-Osorio model. Numbers of nests in which the  
13 parasite egg was rejected or accepted or in which means are calculated are denoted on top of the  
14 bars.

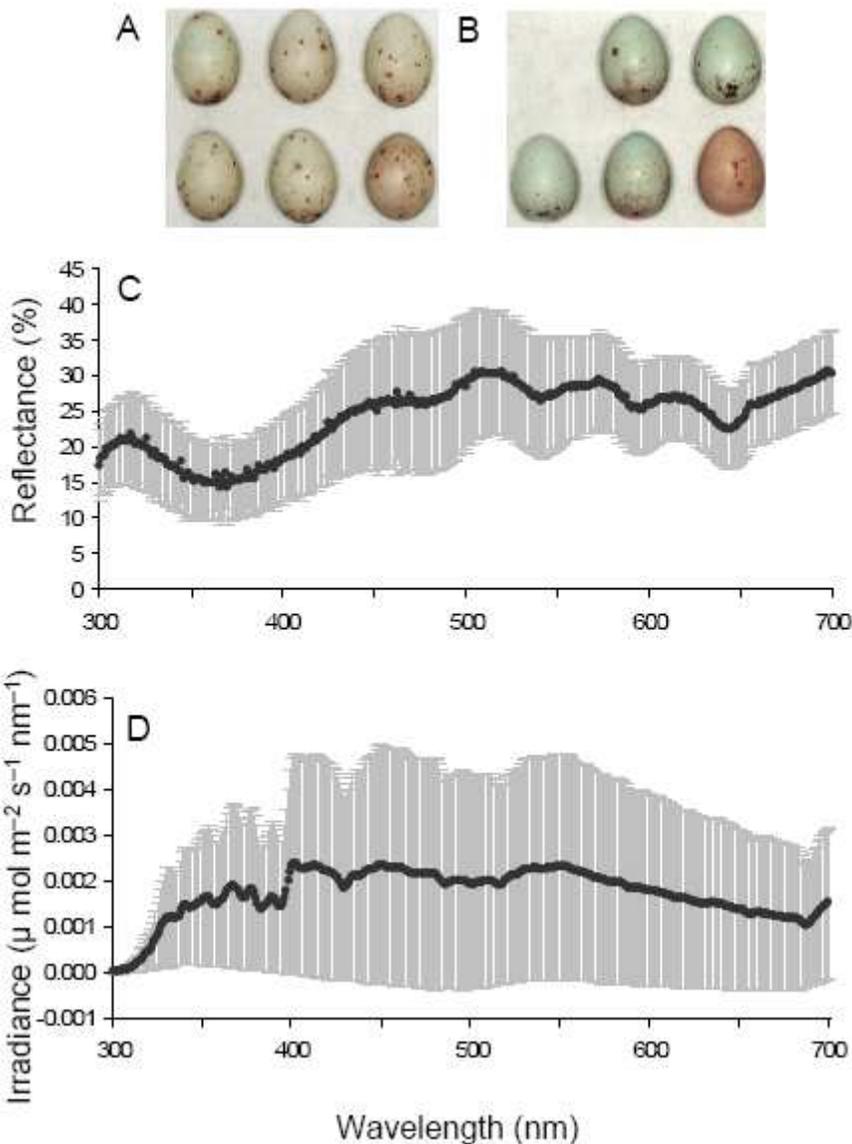
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16

1 **Figure 1:** Avilés et al.

2

3



4

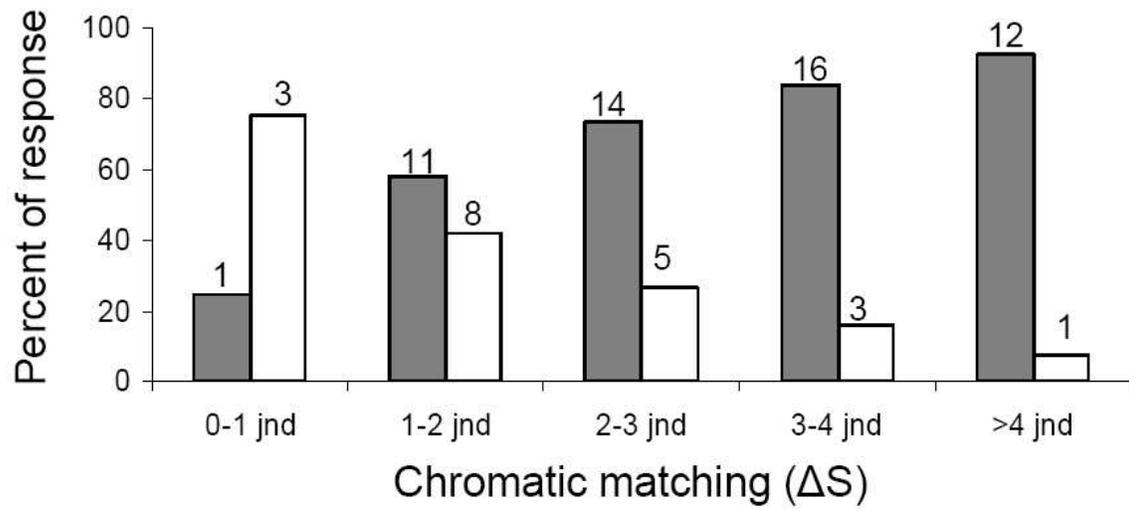
5

1 **Figure 2:** Avilés et al.

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Dear Dr Coltman,

Please find enclosed the revised version of the manuscript entitled “Avian color perception predicts behavioral responses to experimental brood parasitism in chaffinches” (JEB ms JEB-2009-00484)” that you considered accepted provided that we revised it along the lines recommended by the referees.

First of all, we would like to thank the two referees for their constructive comments. Globally the two referees agreed that the main aim of our work (testing for an association between differences in appearance of cuckoo and host eggs as visualized by avian hosts and probability of rejection behaviour) was interesting and satisfactory answered. Referee 1 suggested removing from the manuscript the prediction 2, and all the results and discussion around. The referee 2 also made minor editorial comments. We found all these comments straightforward and thus we have attempted to incorporate fully in the new version hopefully resulting in a significant improvement of the manuscript. Below, we would like to explain one by one how we have dealt with the comments raised by the two referees (*their comments are also given in italics*).

----- **RESPONSES TO REFEREES** -----

***Reviewer: 1***

*This is a very interesting article that for the first time demonstrates an association between differences in appearance of cuckoo and host eggs as visualized by avian hosts and probability of rejection behaviour.*

*I have only one main concern relates to the second prediction analysed. The relative larger inter-clutch variation in relation the intra-clutch variation in egg trait is not necessarily a prediction of the influence of brood parasitism of egg appearance. For instance, it is known that egg colour is related to females' phenotype (quality?) and, for most (all?) species in which egg colour (or other traits of eggs and nestlings) has been estimated, those within the same clutch were of more similar colouration than those from different clutches (i.e egg colour is repeatable within the same clutch). That occur in species that are not under (inter or even intra) brood parasitism selection pressures as flycatchers, starlings and others. In fact that was the reason why author only measure one host egg per nests as exposed in Material an Methods (Pg 7, lines 18-10). The prediction from a brood parasitism scenario driving the evolution of intra-clutch variation is that it should predict detectability and rejection of parasitic eggs. The advantages of clutches with low intra clutch variation will results in a larger inter-clutch variation at the population level. I do not know if author have data to test these predictions but, if it is not the case, I strongly suggest authors removing the text related to intra- inter-clutch variation and the non-exclusive prediction of the former being lower than the later. Under my point of view including this obscure prediction, reduce the general good quality of the manuscript.*

We agree. We have now removed from the manuscript the prediction concerning intra- and inter-clutch variation as well as the parts of the methods, results and discussion sections specifically dealing with that prediction.

*Pg. 3, line 3. Why coadaptation rather than coevolution? I think there are enough evidence to conclude that between cuckoos and host not only exist coadaptation but also coevolution for which evidences in vertebrates are even more scarce.*

We have replaced the term coadaptation by coevolution.

*Pg 3, line 22 - Pg4, line 2. Please develop implications of such limitations that previous studies have assumed. i.e., the use of difference in reflectance between host and foreign eggs as predictor of rejection behaviour included information that might be not perceived by hosts and do not take into account visual environment against the eggs are contrasting. Obviously, estimates of reflectance and visual perception of eggs should be related. However, in the case that egg appearance was in fact associated with probability of detecting and rejecting foreign eggs, these associations would more easily detected with estimates of visual perception. More importantly, egg traits more closely related to visual perception of parasitic egg by host are the traits expected to coevolve with level of egg recognition by hosts.*

We have now developed the arguments as suggested.

*Pg 4, lines 1-6. This paragraph is essential for the understanding of the importance of the paper but, as written is a bit difficult to follow. I recommend rewrite the entire paragraph following the argument in my previous point*

We have rewritten it following referee' advice.

*Pg 5, lines 4-6. The existence of egg types, classified as Fringilla egg morphs, which is a post-hoc human classification is not a very strong evidence of parasitism, but together with the existence of museum clutches of Fringilla parasitized by cuckoo.*

It's true. We have now rewritten the sentence accordingly.

*Pg5, lines 8-11. I do not think it is again necessary to highlight the goodness of the method used. Remove this sentence.*

Done.

*Pg5, line 18, these are not the references suggesting and finding evidences of the role of the intra-clutch variation on rejection behaviour and its consequence on the evolution of the inter-clutch variation of the host population.*

We have now removed this part of the manuscript.

*Pg 5, lines 20-22. This is not necessarily a prediction of the influence of brood parasitism of egg appearance. For instance, it is known that egg colour is related to female phenotype (quality?) and, for most (all?) species in which egg colour has been estimated, those within the same clutch were of more similar colour than those from different clutches (i.e egg colour is repeatable within the same clutch). That occur in species that are not under (inter or even intra) brood parasitism selection pressures as flycatchers, starlings and others. In fact that was the reason why author only measure*

*one host egg per nests as exposed in Material an Methods (Pg 7, lines 18-10)*

We agree and, therefore, removed this part of the manuscript.

*The prediction from a brood parasitism scenario driving the evolution of intra-clutch variation is that it should predict detectability and rejection of parasitic eggs. The advantages of clutches with low intra clutch variation will results in a larger inter-clutch variation at the population level. I do not know if author have data to test these predictions but, if it is not the case, I strongly suggest authors removing the text related to intra- inter-clutch variation and the non-exclusive prediction of the former being lower than the later variation in a context of selection for foreign egg recognition (and associated result, method and discussion). Under my point of view including this obscure prediction, reduce the general good quality of the manuscript.*

Done.

*Pg 7, lines 15-16. How long time were the egg out of nests?. Did affect probability of successful hatching? Information of the implications of experimental procedure is necessary*

We have now clarified that we only measured one egg per clutch and that the egg collected for measurements was used as the experimental parasitic egg in a subsequent experiment.

*Pg 7, lines 18-10: This fact invalidate the importance of predictions dealing with inter-clutch variation. It is already know that it is the case in this and in another species (see comment above)*

We have already removed this part of the manuscript.

*Pg 10, lines 7-18. Well done!*

Thanks.

*Pg 11, lines 13-15. Were these absolute differences estimated for the whole host clutch or with respect to the randomly selected host egg for colour measures? Please, clarify*

We have now clarified that these measures were collected from one randomly selected egg in the host clutch.

*Pg 13, line 23. "variance method". What is it? As far as I can see it is a one-way ANOVA.*

We have already removed this part of the manuscript. In any case, one-way ANOVA is a "variance method" too.

*Pg 14, line 14. "%BI". This index should be very close (inversely) related to repeatability estimation of egg appearance. I agree with author that this index should be related to average differences between experimental and host eggs and, therefore,*

*should predict probability of egg recognition and rejection, but THIS IS EXACTLY THE HYPOTHESIS TESTED HERE with the new methodology. I cannot see the interest of these estimations and testing in the context of the present manuscript. Again I suggest to remove this part of the manuscript*

Done.

*Pg 14, line 1-9. Why are the spectral data used here? The interesting traits, as the author clearly explained in the introduction, are those related to the avian perception of egg appearance and not the spectra. Thus, estimations of intra-clutch variation should not be estimated in that way (isn't it?). Thus, consistently with the authors' reasoning of the importance of the paper, visualization of eggs against the nest background should be used here. Again, I see that reasoning around the estimation and analyses of inter- and intra clutch variation is isolate and obscure the main message of the article. I definitely suggest eliminating it.*

We have removed this analysis that corresponded with the prediction 2.

*Pg. 15 line 17 - Pg. 16 line 2. These are just estimates of something like within-clutch repeatability of egg traits. These estimates are therefore useful for discussing the use of a randomly chosen egg per clutches in the analyses, but not in context of brood parasitism study in a single population. This index should be related to among-populations variation in parasitism selection pressure and/or rejection rates, which is not the case here. Again, remove this section or include this result in the material an method section supporting the use of egg per clutch.*

We have now removed it.

*Pg 15, lines 21-23. Is it a problem for using one egg per clutch? In material and Method you said that egg colour is repeatable within clutches, but it does not look the case for chromatic component!*

Please, see that for PC2 scores, that account for most of the chromatic variation, we found that among clutches variance in chroma of eggs was significantly larger than the within clutch variance (one-way ANOVA,  $F = 2.54$ ,  $df = 17,18$ ,  $P = 0.028$ ; repeatability = 44.0 %). The same apply for the achromatic component of egg coloration (PC1 scores, one-way ANOVA,  $F = 3.58$ ,  $df = 17,18$ ,  $P = 0.0005$ ; repeatability = 57.0 %). So our statement is well founded. In any case, we have now removed this part of the manuscript and do not report this result because, in such a case, we should also extent to much the method section to explain PC calculation on spectrophotometer measures.

### **Reviewer: 2**

*I found this study the most interesting of the work that I know on sensory signals involved in egg coloration and egg mimicry. Overall this work appears to be of a high standard. The experimental system is very neat and the conclusions are convincing. The application of models of sensory signals is exemplary (though I cavil slightly at the term 'perceptual'). I am especially impressed by the measurements of spatial pattern (e.g.*

*spot shape), for which this is no agreed or simple method.*

*The fact that chaffinches lay variable eggs is intriguing, and though it may have a trivial explanation raises questions (also relevant to other types of mimicry). It would be interesting to know whether parasitism affects the level of variation - one can think of reasons why it might either favour or disfavour variability that have implications for chaffinch perception/cognition.*

We agree that knowing to what extent variation in host egg appearance is due to brood parasitism is a major evolutionary question that deserves further study. However, we concur with referee 1 that we can not offer a conclusive answer with our current data. Different selective forces and/or constraint than brood parasitism may have shaped current chaffinch egg appearance and morphology and we do not have an appropriate control for those possibilities in our study. Thus, we have removed the part of the manuscript dealing with variation of egg traits, and focused the manuscript on discrimination as perceived by hosts, where we can provide a sound first test of behavioural responses to natural variation in parasite eggs appearance as it is perceived by hosts.

*There are a very few typos.*

*p.13; line 19 - 'eliminates'*

Done.

*p. 16; l. 18. I would say 'magnitude of the stimulus' not 'intensity' nor 'perceived'. (also l. 20)*

Done.

*p. 17. l.13. 'excluded' not 'discarded'. e.g 'We cannot exclude the possibility that the measure of spottiness .'*

Done.

**-----END OF RESPONSES TO REFEREES-----**

We look forward to hearing about your final decision.

Thank you very much.

Sincerely yours,

Jesús Miguel Avilés  
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Table 1. Pearson's correlations among predictors of rejection and latency to rejection of parasitic eggs (N =74). DeltaS= differences in chromatic contrast between parasite and host eggs as estimated by Vorobyev & Osorio model, DeltaQ= differences in achromatic contrast between parasite and host eggs as estimated by Vorobyev & Osorio model, volume= differences in egg volume between parasite and host eggs; shape= differences in egg shape between parasite and host eggs; spot coverage=differences in coverage of spottiness between parasite and host eggs; spot P/A ratio=differences in P/A ratio of spottiness between parasite and host eggs; spot circularity=differences in circularity of spots between parasite and host eggs. See methods for further details.

	DeltaS	DeltaQ	volume	shape	spot coverage	spot P/A ratio
DeltaQ	.6172 N=74 p=.000					
volume	.1319 N=74 p=.263	.2599 N=74 p=.025				
shape	-.0505 N=74 p=.669	.1639 N=74 p=.163	-.0978 N=74 p=.407			
spot coverage	-.0359 N=74 p=.761	-.1894 N=74 p=.106	-.1784 N=74 p=.128	-.2229 N=74 p=.056		
spot P/A ratio	-.0918 N=74 p=.437	-.0081 N=74 p=.946	-.2729 N=74 p=.019	-.0880 N=74 p=.456	.2097 N=74 p=.073	
spot circularity	-.0431 N=74 p=.715	-.1983 N=74 p=.090	.0492 N=74 p=.677	-.0068 N=74 p=.954	.0562 N=74 p=.635	-.1173 N=74 p=.320

Figure 1. Spot data acquisition. Spots were marked with threshold tools in the binary editor of NIS (NIS-Elements BR 3.0, Nikon Corporation, Amstelveen, The Netherlands). Measurements were delimited to a rectangle of the egg surface. The sample rectangular area was set in such a way as to cover as much as possible of the egg area. However, because we cannot optimize both sides of the rectangle at the same time, we gave priority to the axis along which spotting pattern varies more. In general, passerine eggs vary more along their long axis. The sample rectangle's length was fixed to 90% of the egg length. Once the length of the rectangle was set, the width was adjusted by the enforcement that the sample rectangle must lay within the egg surface.

