



A sum of its individual parts? Relative contributions of different eggshell regions to intraclutch variation in birds

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The rejection of eggs of brood parasites in several species of hosts is based on cues only at the blunt pole (BP) and not at the sharp pole (SP) of the foreign and own eggshell. We investigated whether intraclutch variation is confined to a specific egg pole in species where the extent of intraclutch variation in the overall egg appearance is known to positively covary with either egg rejection rate or the probability of being parasitized. For the two poles separately, we analysed intraclutch variation of eggshell brightness and blue chroma. We quantified intraclutch variation as the standard deviations of these colour metrics, instead of their coefficients of variation which would represent a statistically flawed approach. Pooling measurements of brightness across the whole egg surface led to statistically non-significant results and masked positive correlations of BP brightness with egg rejection or parasitism risk, respectively. In contrast, patterns of blue chroma were important across the whole egg. Thus, the traditional whole egg ‘averaging’ approach may mask biologically important effects of intraclutch variation when the variation and potential signalling functions of egg appearance are confined to a specific egg part (brightness). However, analyses based on only BP and SP eggshell region specific data may also lack the power to detect effects of phenotypic traits that do not vary between egg poles (blue chroma). We advocate the use of a combination of region-specific and whole-eggshell based colour metrics and manipulations in cognitive, perceptual, and ecological studies of foreign egg rejection.

Avian eggs are known to differ greatly in their appearance between species and higher taxonomic levels (Kilner 2006). Yet, typically, intraclutch variation in egg appearance is low – the eggs laid by individual females look remarkably similar within the clutch (Cassey et al. 2009).

Functionally, low intraclutch variation in birds’ egg appearance might play at least two adaptive roles. First, in avian host–parasite coevolutionary systems, reduced colour variation within clutches could facilitate the rejection of foreign eggs laid by con- and/or heterospecific brood parasites (Soler and Møller 1996, Moskát et al. 2008). Second, egg colour variation within a clutch might affect the probability of being parasitized by brood parasites (Poláčiková et al. 2009).

Irrespective of the exact function, similarities of egg traits in previous studies of host–parasite coevolution were studied using mean trait (colour, maculation, reflectance spectra) values per each host egg when evaluating egg similarities within clutches. Yet, in most small passerines, maculation or speckling is concentrated at the blunt egg pole (hereafter BP) whereas the sharp egg pole (hereafter

SP) is usually covered with very few or even no spots (Kilner 2006, Poláčiková and Grim 2010). Thus, averaging heterogeneous regions across the whole egg surface might be misleading both with respect to characterising egg phenotype and understanding the salience of each egg region’s appearance regarding the discrimination of own vs foreign eggs by hosts (Poláčiková et al. 2007, Honza and Poláčiková 2008). Indeed, recent studies showed that various avian species use only cues at BP, but not those at SP, when rejecting foreign eggs (Lahti and Lahti 2002, Poláčiková et al. 2007, 2010, Poláčiková and Grim 2010). Therefore, it is necessary to address whether phenotypic variation between eggs is confined to specific parts of eggshells.

Investigating each egg pole separately, we asked whether the averaging of the measurements across the whole egg surface (as done in all previous studies) is sufficient to identify the role of intraclutch variation in egg appearance. We studied species in which intraclutch variation across the whole egg had already been shown to: 1) influence behavioural responses to experimentally added conspecific

eggs (blackcaps *Sylvia atricapilla*; Polačiková et al. 2007), or 2) reflect the probability of being parasitized by common cuckoo *Cuculus canorus* (great reed warblers *Acrocephalus arundinaceus*; hereafter warbler; Polačiková et al. 2009). In both species, behavioural evidence demonstrates that both rely on BP when rejecting foreign eggs (Polačiková et al. 2007, Polačiková and Grim 2010). Therefore, we predicted that components of intraclutch variation influencing parasite egg recognition are related to the appearance of BP but not SP. We evaluated two physical measures related to eggshell colouration (brightness and blue chroma), because only these attributes had been shown as important parameters in previous studies (Polačiková et al. 2007, 2009).

Methods

In the first dataset we reanalysed previously reported behavioural responses towards experimentally added conspecific eggs in the blackcap (Polačiková et al. 2007). The second dataset was from a study of the probability of being parasitized by common cuckoos in warblers (Polačiková et al. 2009). Eight non-parasitized clutches from the latter work were excluded from the present analyses because the different egg poles were not measured separately (see below).

The eggs were measured by a reflectance spectrophotometer (USB2000, Ocean Optics) under standard light conditions (Polačiková et al. 2007, 2009). Each egg was divided into three regions across the longitudinal axis. Each region comprised a third of the length of the egg representing BP, medium egg part (hereafter MP) and SP. From each egg part three randomly sited measurements were taken (each covering $\sim 1 \text{ mm}^2$). In both prior published studies (see above), the mean value from all nine measurements per whole egg surface (hereafter WS) was used when calculating intraclutch variation. Here we calculated mean values for BP, MP and SP separately.

For each egg part separately, we obtained total reflectance for the 325–700 nm range of the light spectrum. We calculated two variables of eggshell colour: brightness (sum of the total reflectance values for all the UV and visible wavelength intervals: $R_{325-700}$) and blue chroma (ratio of the reflectance sums $R_{400-475}/R_{325-700}$). We chose to analyse only these two eggshell colour characteristics because both variables have been previously shown to affect egg recognition in blackcaps (Polačiková et al. 2007), and intraclutch variation in blue chroma is correlated with the probability of being parasitized in warblers (Polačiková et al. 2009).

Previous studies used the coefficient of variation (CV) to estimate variability in particular parts of reflectance spectra, but such approach is statistically flawed (Dale 2006). Therefore, we used standard deviation (SD) instead of CV (as recommended by Dale 2006), so that the results were comparable between ‘whole egg’ vs ‘egg pole’ analyses, we also reanalysed data from Polačiková et al. (2007, 2009) using SD as a measure of spectral variation for WS.

Data analyses

We analysed data for blackcaps and warblers separately. In blackcaps ($n = 44$), the SD of MP measurements did not

correlate with any of the two egg poles in brightness or with BP blue chroma (all $r < 0.13$, $p > 0.41$). In contrast, MP was positively correlated with SP blue chroma ($r = 0.36$, $p = 0.02$). In warbler ($n = 57$), MP was positively correlated with both egg poles for brightness (MP vs BP: $r = 0.32$, $p = 0.01$; MP vs SP: $r = 0.29$, $p = 0.03$), but not for blue chroma (both $r < 0.17$, $p > 0.22$). Given that we had no specific a priori predictions for MP we included only BP and SP data in further analyses.

We employed model selection based on AIC_c and focused on biologically relevant information (effect sizes) instead of p-values (Burnham and Anderson 2002). The SD of brightness and blue chroma were not significantly correlated between BP vs SP in either blackcaps (brightness $r = -0.13$, $p = 0.39$; blue chroma $r = 0.20$, $p = 0.19$, $n = 44$), or warblers (brightness $r = 0.23$, $p = 0.09$; blue chroma: $r = 0.12$, $p = 0.36$, $n = 57$). However, the two colour parameters calculated for both egg poles correlated positively with those averaged for WS (all $r > 0.28$, $p < 0.03$). Therefore, it would be erroneous to include WS and any pole-specific data in one model due to the problem of multicollinearity. Thus, we considered four candidate models (Table 1).

We square-root transformed the intraclutch variation before the analyses to normalize residual variation following Stokke et al. (2002; Shapiro–Wilk tests for transformed variables, all $W > 0.96$, all $p > 0.07$). Results are presented as means \pm SE if not stated otherwise. Statistical analyses were conducted in R 2.11.1.

Table 1. Binomial logistic regression models of colouration of different egg parts (WS = whole egg surface; BP = blunt pole; SP = sharp pole) on the probability of egg rejection in blackcaps ($n = 44$) and being parasitized in great reed warblers ($n = 57$). All SD of colour variables were square-root transformed to achieve normality of error variance. Models are ranked according to AIC_c . Top candidate models ($\Delta AIC_c < 2$) in bold.

Model	K	AIC_c	ΔAIC_c	Weight
Blackcap				
Brightness:				
BP	2	53.3	0.0	0.71
BP+SP	3	55.2	1.9	0.27
BW	2	61.8	8.5	0.01
BS	2	62.0	8.7	0.01
Blue chroma:				
WS	2	54.7	0.0	0.77
SP	2	57.9	3.2	0.15
BP+SP	3	60.1	5.4	0.05
BP	2	61.5	6.9	0.02
Great reed warbler				
Brightness:				
BP+SP	3	74.3	0.0	0.57
BP	2	75.6	1.3	0.29
SP	2	77.5	3.2	0.11
WS	2	80.6	6.3	0.02
Blue chroma:				
WS	2	73.1	0.0	0.96
BP	2	80.3	7.2	0.03
BP+SP	3	82.3	9.2	0.01
SP	2	82.7	9.6	0.01

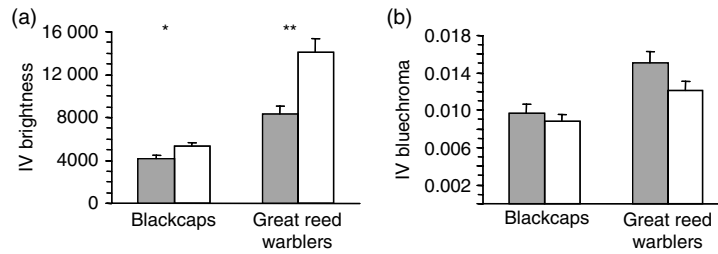


Figure 1. The differences in intraclutch variation IV (SD) in brightness (a) and blue chroma (b) between blunt (gray bars) and sharp poles (open bars) of the eggs (blackcaps: $n = 44$, great reed warblers: $n = 57$). Shown are means + SE for raw untransformed data. * $p < 0.05$, ** $p < 0.001$.

Results

In both species, BP had significantly (paired t -tests) lower intraclutch variation in brightness than SP (blackcap: $t_{43} = -2.24$, $p = 0.03$; warbler: $t_{56} = 4.86$, $p < 0.001$; Fig. 1a). In contrast, egg poles did not differ significantly in blue chroma in either blackcaps ($t_{43} = 0.56$, $p = 0.58$), or warblers ($t_{56} = 1.87$, $p = 0.07$; Fig. 1b).

In blackcaps, rejecters had lower intraclutch variation in brightness at BP than acceptors (Fig. 2a). In contrast, blue chroma at neither BP nor SP covaried with egg rejection, but WS did so (Fig. 2b). Although the best models for brightness contained both BP and SP (Table 1), model averaged estimates showed that 95% CIs for SP contained zero (-0.02 to 0.01) and BP remained the sole predictor of rejecter status (effect size = -0.07 ± 0.03 ; CI -0.13 to -0.02). The most parsimonious model for blue chroma contained only WS (Table 1; effect size = -58.2 ± 24.6).

In warblers, parasitized females had lower intraclutch variation in brightness at BP than non-parasitized ones (Fig. 2c). In contrast, blue chroma at either BP or SP was unrelated to parasitism status of warbler females (Fig. 2d).

Although the best models for brightness contained both BP and SP (Table 1), model averaged estimates showed that 95% CIs for SP contained zero (-0.03 to 0.01) and BP remained the sole predictor of parasitism status (effect size = -0.03 ± 0.01 ; CI -0.05 to -0.003). The most parsimonious model for blue chroma contained only WS (Table 1; effect size = -32.5 ± 11.4).

Discussion

Previous studies (testing both egg poles separately), based on analyses of clutch photographs and human visual assessment, suggested that within-clutch variation might be confined mainly to the blunt egg pole traits (Lahti and Lahti 2002, Spottiswoode and Stevens 2010). Here, using objective spectral reflectance data, we confirmed that intraclutch variation in egg brightness was significantly lower at BP than at SP in both study species. In contrast, intraclutch variation in blue chroma was similar at BP and SP in both blackcaps and warblers. Averaging brightness across the whole egg surface masked the experimentally evoked behavioural effects of BP specific brightness,

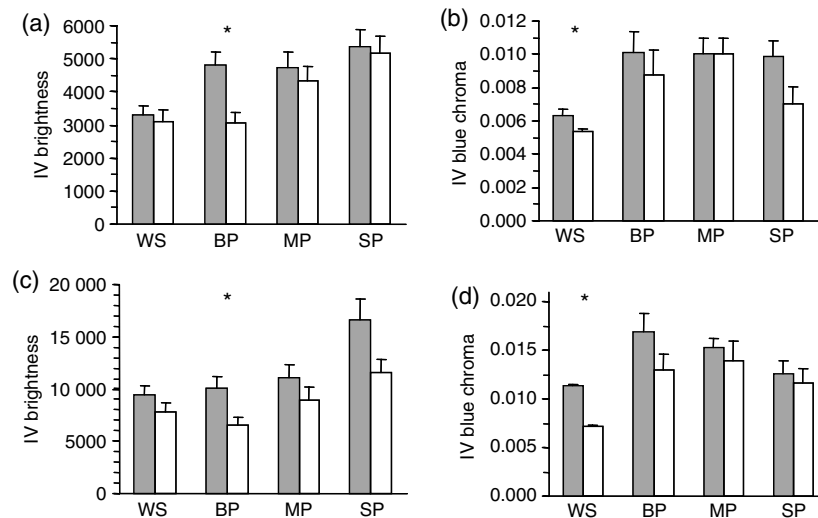


Figure 2. Intraclutch variation (IV) according to species, colour parameter and egg part. Variation in brightness (a, c) and blue chroma (b, d) was measured as a standard deviation (SD) per each clutch at whole egg surface (WS), blunt pole (BP), medium portion (MP) and sharp pole (SP) of the egg. The two upper panels (a, b) show data for clutches in which blackcaps accepted (full bars, $n = 28$) or rejected (open bars, $n = 16$) parasite eggs. The two lower panels (c, d) show data for great reed warbler clutches that were not parasitized (full bars, $n = 29$), or were parasitized (open bars, $n = 28$) by common cuckoos. Shown are means + SE for raw untransformed data. Results of Welch t -tests; * $p < 0.05$.

whereas averaging blue chroma revealed behavioural effects of this parameter that were not detected with analyses of BP or SP separately.

We suggest that the analysis of both egg poles might be critical when evaluating the intraclutch variation in egg appearance with respect to behavioural and ecological predictors of host responses in the context of host–parasite eggshell mimicry. In many passerines, the speckling (maculation) is usually focused at egg blunt poles and might serve as a female’s ‘fingerprint’ in foreign egg recognition (Kilner 2006). Among the many studies aimed at testing the prediction that lower host intraclutch variation facilitates identification of parasitic eggs only few supported this hypothesis at the intraspecific level (reviewed by Moskát et al. 2008), whereas, contrary to predictions, Avilés et al. (2004) and Cherry et al. (2007) revealed higher intraclutch variation in rejecters than in acceptors. This inconsistency in findings across the studies has at least two explanations: 1) previous studies experimentally parasitized only naturally non-parasitized host pairs, which could be mainly younger/older breeders with higher intraclutch variation compared to older parasitized birds with uniform clutches (Polačiková et al. 2009) or, 2) these studies used only mean values per each egg surface when testing for within clutch variation. This ‘averaging’ approach may have masked existing effects of intraclutch variation as demonstrated in our analyses.

Contrasting results for brightness vs blue chroma can be explained by varying patterns of within-egg variation in brightness vs hue (Polačiková and Grim 2010). Brightness is heterogeneous within egg (it is lower at BP than at SP) whereas hues are distributed homogeneously across the eggshell (Polačiková et al. 2007). Therefore, pooling of brightness values from BP and SP may mask perceptually relevant effects of brightness that are confined to BP. In contrast, separating blue chroma data between two poles may decrease the power of the comparison and make effects statistically non-significant (although the direction of the patterns remain the same trends, Fig. 2d).

In sum, reduced intraclutch variation in specific regions of host eggs, which are also important in the context of brood parasite-host interactions, can be confined to BP (cf. Polačiková and Grim 2010, Polačiková et al. 2010). We suggest that future spectrophotometric studies should relate the appearance of specific regions of eggs to behavioural responses to natural and manipulated eggshell appearance in those regions.

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