

## RESEARCH ARTICLE

# The role of egg–nest contrast in the rejection of brood parasitic eggs

Zachary Aidala<sup>1,2,3,\*</sup>, Rebecca Croston<sup>4,5</sup>, Jessica Schwartz<sup>2</sup>, Lainga Tong<sup>2</sup> and Mark E. Hauber<sup>1,2,4</sup>

## ABSTRACT

Hosts of avian brood parasites can avoid the reproductive costs of raising genetically unrelated offspring by rejecting parasitic eggs. The perceptual cues and controls mediating parasitic egg discrimination and ejection are well studied: hosts are thought to use differences in egg color, brightness, maculation, size and shape to discriminate between their own and foreign eggs. Most theories of brood parasitism implicitly assume that the primary criteria to which hosts attend when discriminating eggs are differences between the eggs themselves. However, this assumption is confounded by the degree to which chromatic and achromatic characteristics of the nest lining co-vary with egg coloration, so that egg–nest contrast per se might be the recognition cue driving parasitic egg detection. Here, we systematically tested whether and how egg–nest contrast itself contributes to foreign egg discrimination. In an artificial parasitism experiment, we independently manipulated egg color and nest lining color of the egg-ejector American robin (*Turdus migratorius*), a host of the obligate brood parasitic brown-headed cowbird (*Molothrus ater*). We hypothesized that the degree of contrast between foreign eggs and the nest background would affect host egg rejection behavior. We predicted that experimentally decreasing egg–nest chromatic and achromatic contrast (i.e. rendering parasitic eggs more cryptic against the nest lining) would decrease rejection rates, while increasing egg–nest contrast would increase rejection rates. In contrast to our predictions, egg–nest contrast was not a significant predictor of egg ejection patterns. Instead, egg color significantly predicted responses to parasitism. We conclude that egg–egg differences are the primary drivers of egg rejection in this system. Future studies should test for the effects of egg–nest contrast per se in predicting parasitic egg recognition in other host–parasite systems, including those hosts building enclosed nests and those parasites laying cryptic eggs, as an alternative to hypothesized effects of egg–egg contrast.

**KEY WORDS:** Brood parasitism, Visual modeling, Visual ecology, Egg rejection

## INTRODUCTION

Obligate brood parasites circumvent the costs of parental care and lay their eggs in the nests of other species (Davies, 2000). By

accepting the burden of raising genetically unrelated offspring, brood parasite hosts suffer major fitness costs (Øien et al., 1998; Lorenzana and Sealy, 2001; Hauber, 2003a,b; Hoover, 2003). The rejection of foreign eggs in the nest is an effective defense against brood parasitism (Rothstein, 1975; Grim et al., 2011; Kilner and Langmore, 2011), which places reciprocal selective pressure on parasites to evolve egg coloration and/or maculation to match that of its host. This then selects for increasingly fine-tuned discrimination by hosts (Davies and Brooke, 1989; Stoddard and Stevens, 2010, 2011; Davies, 2011). Such an arms-race is a canonical example of co-evolutionary processes driving both perceptual and signaling mechanisms (Davies and Brooke, 1989; Davies, 2011; Igic et al., 2012; Stoddard et al., 2014).

The proximate, perceptual controls underlying egg rejection behavior have been intensively studied in various brood parasite–host systems (Kilner and Langmore, 2011). Generally, an egg should be perceived as foreign if it differs beyond a given threshold from the variation present within a host female’s natural clutch (Reeve, 1989; Rodríguez-Gironés and Lotem, 1999). Such recognition is dependent on a number of factors, including the population parasitism rate (Davies et al., 1996), the number of host eggs present, and the timing of egg parasitism (e.g. Moskát and Hauber, 2007). Hosts’ acceptance thresholds also vary according to experience, even within a single clutch (Hauber et al., 2006). Hosts can respond to differences in eggshell background color (Avilés et al., 2005, 2010; Honza et al., 2007; Honza and Polačiková, 2008; Moskát et al., 2008; Bán et al., 2013; Croston and Hauber, 2014a), maculation pattern (Lawes and Kirkman, 1996; Lahti and Lahti, 2002; López-de-Hierro and Moreno-Rueda, 2010; Spottiswoode and Stevens, 2010), egg brightness (Lahti, 2006; Gloag et al., 2014), egg size (Rothstein, 1982; Marchetti, 2000) and egg shape (Guigueno and Sealy, 2012) when discriminating their own from foreign eggs.

While above-threshold visual contrast is increasingly known to induce egg rejection among brood parasite hosts, it is not firmly established whether comparing own versus foreign eggs is a more reliable cue than other visual comparisons available in the host’s nest environment (Endler and Mielke, 2005; Thorogood and Davies, 2013). For example, relatively few studies have examined whether and how nest lining color influences parental behavior (but see Bailey et al., 2015). Regarding parasitic egg rejection by host parents, the role of egg–nest contrast has similarly not been well established (Siefferman, 2006), and only a handful of studies have experimentally tested the hypothesis that visual contrasts between eggs and their background (i.e. the nest lining) affect egg rejection decisions (Gloag et al., 2014; Honza et al., 2014). Growing evidence suggests that there is selective pressure for brood parasites to evolve dark, cryptic eggs among Australasian cuckoo–host systems, making egg detection by hosts or, rather, by competing parasites difficult because the eggs blend in with the nest background (Langmore et al., 2005, 2009; Gloag et al., 2014). While similar arguments have also been made for other host–parasite systems (Mason and Rothstein,

<sup>1</sup>Biopsychology and Behavioral Neuroscience Subprogram in Psychology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, NY 10016-4309, USA. <sup>2</sup>Department of Psychology, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065, USA. <sup>3</sup>Social and Behavioral Sciences Division, Bloomfield College, Bloomfield, NJ 07003, USA.

<sup>4</sup>Ecology, Evolutionary Biology, and Behavior Subprogram in Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, NY 10016-4309, USA. <sup>5</sup>Department of Biology – Program in Ecology, Evolution, and Conservation Biology, University of Nevada – Reno, Reno, NV 89503, USA.

\*Author for correspondence (Zachary\_aidala@bloomfield.edu)

1987; Honza et al., 2011, 2014), experimental tests of whether egg–nest contrast affects parasitic egg discrimination in the context of both natural and experimental egg color variation are lacking.

We focused on the North American brown-headed cowbird [*Molothrus ater* (Boddaert 1783); hereafter, cowbird]–American robin (*Turdus migratorius* Linnaeus 1766; hereafter, robin) parasite–host system. Robins are a suitable study species in that they are one of fewer than 30 documented cowbird host species to eject cowbird eggs at rates above 75% (Briskie et al., 1992; Peer and Sealy, 2004), allowing for the testing of specific sensory hypotheses mediating egg rejection in this system. Previous work on this species pair showed that natural and model cowbird eggs are perceptually distinct from natural (conspecific) robin eggs: natural parasite eggs are rejected from 100% of experimental nests, whereas conspecific natural and model robin eggs are not rejected (Briskie et al., 1992; Croston and Hauber, 2014a; Rothstein, 1982; Fig. 1A). Because egg color variability within robin clutches is significantly lower than egg color variability between clutches, robins may compare foreign eggs against the relatively low color variability present within the entire clutch in their egg rejection decisions (Abernathy and Peer, 2014; Croston and Hauber, 2015; see also Fig. 1B).

In this host–parasite system, artificially colored and natural eggs also exhibit strongly and positively correlated chromatic contrast against both natural robin eggs and natural robin nest linings, as measured by avian visual modeling (Fig. 1B). Similar to robins' intra-clutch color variability, natural robin nest linings show low

spectral variability across the avian visible range (supplementary material Fig. S1) as well as low avian-perceived chromatic and achromatic contrasts when compared against each other (supplementary material Fig. S2). Further, avian-perceived visual chromatic and achromatic contrasts between robin eggs and natural nest linings are generally lower than that between cowbird eggs and robin nests (Fig. 1B). Thus, egg–nest contrast potentially confounds the degree to which we understand egg–egg contrasts to serve as the necessary and/or sufficient cues for parasitic egg discrimination in this and other host–parasite systems.

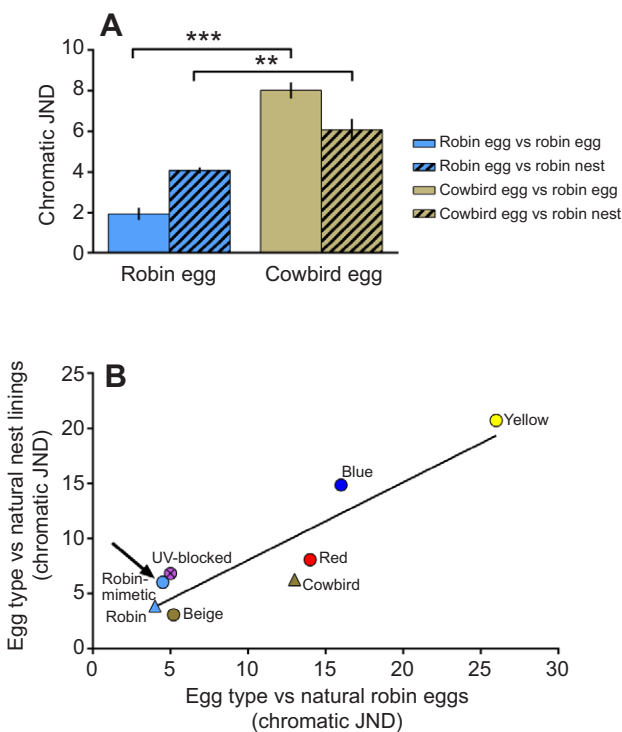
Here, we hypothesized that artificial eggs that more closely resemble the nest background (i.e. are cryptic) are more likely to be accepted. We experimentally tested the degree to which egg–nest contrast affects egg rejection, independent of egg–egg contrast, predicting that increasing/decreasing egg–nest contrast (thereby rendering eggs less/more cryptic), would increase/decrease parasitic egg ejection rates. Alternatively, egg–nest contrast may not itself affect hosts' rejection decisions, which would support the role of foreign versus own egg differences themselves as the primary cue for parasitic egg discrimination. To establish the degree to which egg–nest contrast per se influences parasitic egg discrimination, we manipulated the nest-lining color of robin nests (Fig. 2) in an artificial brood parasitism experiment.

We parasitized robin nests with plaster-of-Paris eggs painted the same colors as our nest lining manipulations (cowbird ground color-mimetic – hereafter, beige; blue–green – hereafter, robin-mimetic; and red), and whose rejection rates in non-manipulated nests are known from our published work (Table 1, Fig. 2; these egg colors and their rejection rates in natural nests were sourced from Croston and Hauber, 2014a). To determine the extent to which we successfully manipulated artificial egg–nest lining contrast, we conducted avian visual modeling analyses on egg and nest-lining reflectance spectra (Fig. 3), and analyzed raw reflectance spectra themselves (see Materials and methods) as a methodological check. We specifically predicted that artificially increasing the visual contrasts (measured as just-noticeable differences, or JNDs, from visual modeling analyses) between experimental parasitic eggs and the nest background would result in increased rejection rates, while artificially decreasing contrast would decrease rejection rates (Table 1). We then tested our predictions by assessing the extent to which artificial egg–nest lining achromatic and chromatic contrasts predicted egg rejection rates.

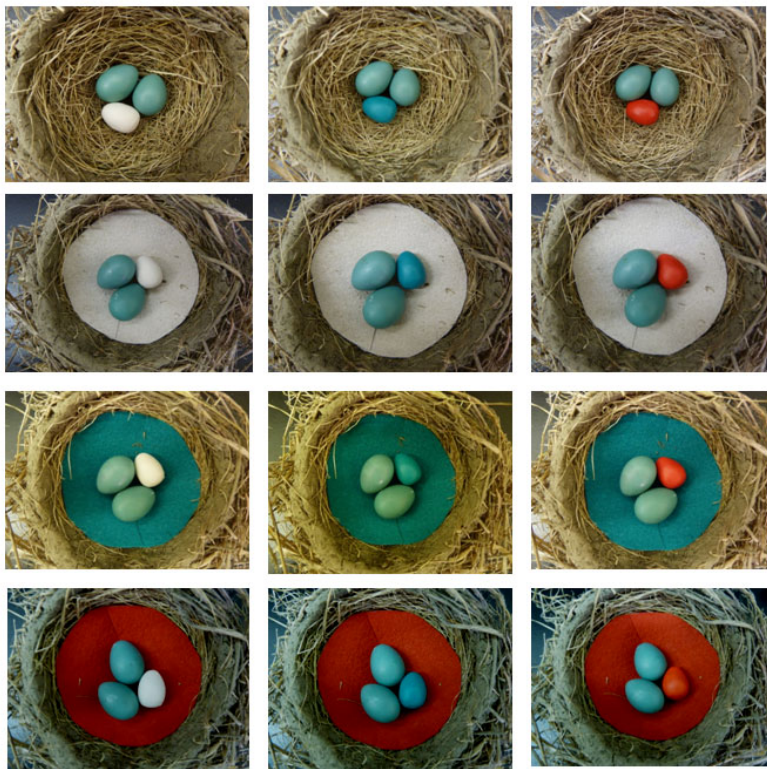
## RESULTS

### Covariation of egg–egg versus egg–nest contrasts with published egg rejection rates

Natural robin eggs (which elicit no ejection; Briskie et al., 1992) possessed significantly lower chromatic contrasts than natural cowbird eggs (which are always ejected) when compared with natural robin eggs sourced from different conspecific nests ( $U_1=21.77$ ,  $P<0.0001$ ; Fig. 1A). In parallel, natural robin eggs possessed significantly lower chromatic contrasts against natural robin nest linings relative to natural cowbird eggs ( $U_1=6.00$ ,  $P=0.01$ , Fig. 1A). Further supporting our claim that there is a quantitative confound between egg–egg chromatic contrast and egg–nest chromatic contrast, we found a strong and significant positive relationship between artificial egg–natural robin egg and artificial egg–natural nest chromatic JNDs ( $F_{1,4}=30.24$ ,  $P=0.0053$ ; Fig. 1B) by including natural and artificial egg stimuli analyzed in Croston and Hauber (2014a). We also found that color variation among natural robin nest linings is low (supplementary material Figs S1 and S2), suggesting that the nest lining itself presents a reliable cue to be used by robins to perceptually discriminate own from foreign eggs.



**Fig. 1. Chromatic contrasts differentiating robin eggs from cowbird eggs, and robin eggs from natural (unmanipulated) robin nests.** (A) Comparison of chromatic contrasts of robin eggs, which elicit no ejection (Briskie et al., 1992), and cowbird eggs, which elicit 100% ejection by robins, against robin eggs and natural robin nest linings. Conspecific robin eggs had significantly lower chromatic contrasts than cowbird eggs against both robin eggs ( $U_1=21.77$ ,  $***P<0.0001$ ) and natural nest linings ( $U_1=6.00$ ,  $**P=0.01$ ). (B) Linear regression between mean egg–egg and egg–nest chromatic just noticeable differences (JNDs) for artificial egg types ( $F_{1,4}=30.24$ ,  $P=0.0053$ ) using combined data from Croston and Hauber (2014a). For comparison, natural robin and cowbird egg chromatic contrasts are also shown.



**Fig. 2. Artificial eggs in natural (top row), beige (second row), robin-mimetic (third row) and red (bottom row) nests.** Artificial eggs were constructed of painted plaster-of-Paris (measuring 21×16 mm). Experimental nests were lined with a felt pad (mean disc diameter 94 mm) that was painted with the same paint colors as artificial eggs and affixed to the bottom of American robin nests using fast-drying, non-toxic glue.

### Perceptual outcomes of egg/nest lining color manipulations

We found that natural robin egg–egg chromatic contrasts (mean±s.e.m. 1.91±0.30) were significantly lower than natural egg–natural nest lining contrasts (3.98±0.19;  $U_1=9.60$ ,  $P<0.001$ ; Fig. 1). In contrast, achromatic natural egg–egg contrasts (2.84±0.65) were not significantly different from egg–natural nest lining contrasts (3.70±1.53;  $U_1=0.154$ ,  $P=0.69$ ) when compared with natural nest lining. Together, these results suggest that it is chromatic contrast against the natural nest lining that may provide a strong cue against which to compare foreign eggs.

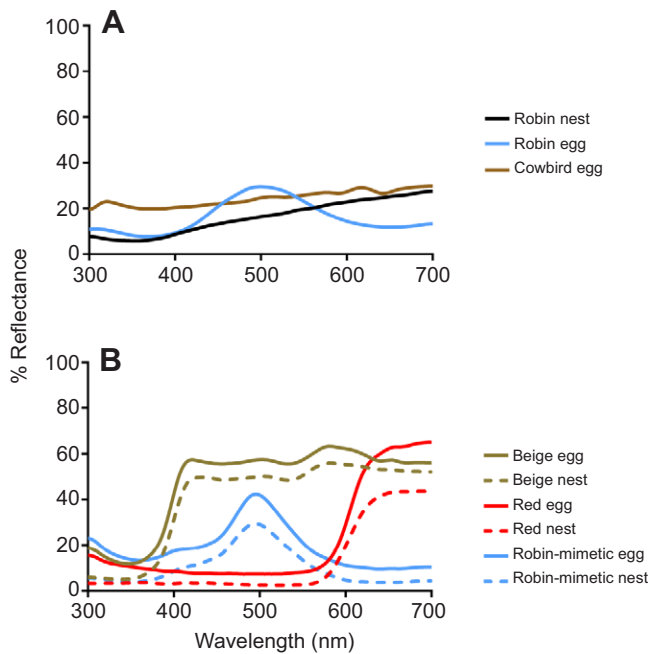
We compared avian-perceived chromatic differences between all eggs and nest-lining colors to test our predictions outlined in Table 1. We found a significant effect of nest-lining color ( $N_{\text{beige nests}}=15$ ,  $N_{\text{red nests}}=15$ ,  $N_{\text{robin-mimetic nests}}=15$ ,  $N_{\text{natural nest}}=5$ )

on chromatic contrast among beige eggs ( $H_3=44.63$ ,  $P<0.0001$ ). All pairwise comparisons were significant ( $P<0.05$ ; Fig. 4A); beige eggs had the highest chromatic contrast in red nests, followed by robin-mimetic nests, natural nests, then beige nests. We also found a significant effect of nest-lining color on chromatic contrast among robin-mimetic eggs ( $H_3=42.67$ ,  $P<0.0001$ ). Red nests had the highest chromatic contrast with robin-mimetic eggs, followed by beige nests, natural nests and robin-mimetic nests. The amount of chromatic contrast between robin-mimetic eggs and nests differed significantly among all pairs ( $P<0.05$ ), except between beige and natural nests (Fig. 4C). Lastly, we found a significant effect of nest-lining color on chromatic contrast among red eggs ( $H_3=45.00$ ,  $P<0.0001$ ). All pairwise comparisons were significant ( $P<0.05$ ;

**Table 1. Experimental nest/egg color manipulations showing the predicted and observed effects on rejection rates in robins**

Nest color	Egg color	Rejection rate in natural nests (%)	Predicted change in rejection rate	Observed change in chromatic contrast	% Experimental eggs rejected	Rejection rate 95% CI	$\chi^2$ test
Beige	Beige	100	Decrease	Decrease	92	0.67–0.99	$\chi^2=24.69$ , $P<0.05$
	Mimetic	0	Increase	No Change	0	0.00–0.30	
	Red	64	Increase	Increase	33	0.14–0.61	
Mimetic	Beige	100	No change	Increase	75	0.41–0.93	$\chi^2=4.68$ , $P=0.10$
	Mimetic	0	No change	Decrease	25	0.07–0.59	
	Red	64	Increase	Increase	67	0.30–0.90	
Red	Beige	100	No change	Increase	73	0.43–0.90	$\chi^2=3.97$ , $P=0.14$
	Mimetic	0	Increase	Increase	30	0.11–0.60	
	Red	64	Decrease	Decrease	55	0.28–0.79	

Observed change in chromatic contrast refers to the predicted change in avian perceivable color contrast for experimental eggs–natural nests relative to experimental eggs–experimental nests. Rejection rates in natural nests (unmanipulated nest lining) were sourced from Croston and Hauber (2014a). Observed rejection rate and 95% confidence interval (CI) of experimental egg colors in each experimental nest lining are also shown.  $\chi^2$  analyses of experimental egg color rejection/acceptance are split by nest lining color; bold indicates significance at  $\alpha=0.05$ , with d.f.=2 for all analyses.



**Fig. 3.** Mean interpolated spectra of natural and artificial eggs and nests used in visual modeling analyses. (A) Natural (unmanipulated) nest linings and natural eggs; (B) artificial eggs. Spectral measurements were taken across the avian-visible range (300–700 nm; see Materials and methods).

Fig. 4E); red eggs in robin-mimetic nests had the highest chromatic contrast, followed by beige nests, natural nests and red nests.

The analyses above were conducted using an ultraviolet-sensitive (UVS) perceptual model for robin vision (based on Aidala et al., 2012), and we carried out a separate set of visual model analyses using violet-sensitive (VS) visual model parameters (see Materials and methods). The results followed the same chromatic contrast patterns as above, although JND values were generally much larger using this model than in our UVS visual model (Fig. 4; supplementary material Fig. S3A,C,E). Similarly, chromatic distance analyses of chromatic principal component (PC)2 and PC3 scores (supplementary material Table S1, Fig. S4) of raw reflectance spectra (as a measure of chromatic distance) between eggs and nest linings corroborated the patterns seen in both of our visual modeling analyses (Fig. 4A,C,E; supplementary material Fig. S5) and followed the same pattern when compared against rejection rates as chromatic JNDs (Fig. 5A; supplementary material Fig. S6A and Fig. S7A).

We also compared avian-perceived achromatic differences between all eggs and nest-lining colors (Fig. 4B,D,F). We found a significant effect of nest-lining color on achromatic contrast among beige eggs ( $H_3=43.54$ ,  $P<0.0001$ ; Fig. 4B). There was also a significant effect of nest-lining color on achromatic contrast among robin-mimetic eggs ( $H_3=38.03$ ,  $P<0.0001$ ; Fig. 4D) and red eggs ( $H_3=41.38$ ,  $P<0.0001$ ; Fig. 4F). Neither our VS visual modeling analysis (supplementary material Fig. S3) nor our PC1 distances (as a measure of achromatic distance – see Materials and methods; supplementary material Fig. S5B,D,F) paralleled the visual contrasts in our achromatic UVS visual model. However, because neither PC1 distances nor achromatic JNDs (from either visual modeling analysis) between artificial eggs and nest linings were significantly related to rejection rate (see below; Fig. 5B, supplementary material Figs S6B and S7B), we only included achromatic JNDs in further behavioral analyses so as to be consistent with our analysis of chromatic JNDs. Because of the similarities in chromatic contrasts

for both the VS visual model and analysis of chromatic principal components, we focused on our primary UVS visual modeling data (JNDs) for our behavioral analyses (see below).

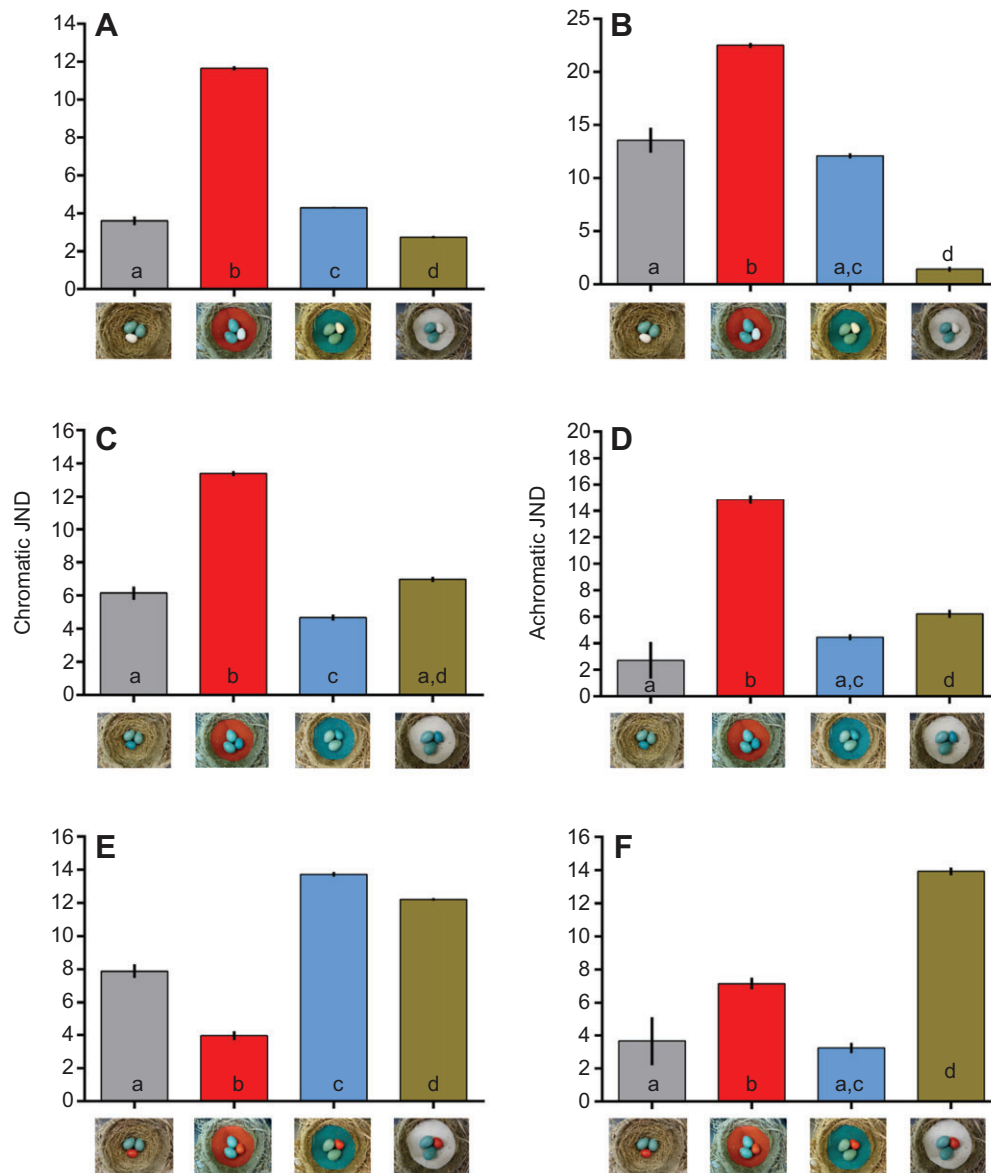
### Behavioral experiments

We conducted a total of 94 artificial parasitism experiments with model eggs ( $N_{\text{beige eggs}}=34$ ,  $N_{\text{robin-mimetic eggs}}=29$ ,  $N_{\text{red eggs}}=31$ ) in nests with beige ( $N=17$ ), red ( $N=19$ ) and robin-mimetic ( $N=12$ ) linings. When combined with egg rejection rate data of artificial egg colors in natural nests from Croston and Hauber (2014a), the mean chromatic contrasts in egg–nest treatments were not significantly related to the rejection rate in our egg–nest manipulations, and the regression slope was slightly negative and thus in the direction opposite to our predictions ( $F_{1,10}=0.49$ ,  $P=0.50$ ,  $R^2=0.05$ ; Fig. 5A). Similarly, when natural nest data were removed from this analysis, the relationship trended in the same direction but remained non-significant ( $F_{1,7}=0.25$ ,  $P=0.63$ ,  $R^2=0.03$ ). Achromatic contrasts were also not significantly related to rejection rate in our nest manipulations both with natural nest rejection data from Croston and Hauber (2014a) included ( $F_{1,10}=0.43$ ,  $P=0.53$ ,  $R^2=0.04$ ; Fig. 5B) and without these data ( $F_{1,7}=0.0004$ ,  $P=0.98$ ,  $R^2<0.01$ ).

A Friedman ANOVA revealed a consistent effect of egg color on robin egg rejection behaviors: relative egg rejection rates were consistently ordered as beige>red>robin-mimetic eggs across, and irrespective of, the three colors of experimental and one natural nest lining types ( $\chi^2_2=8.00$ ,  $P=0.018$ ; Fig. 5C). To confirm these results, we fitted binomial generalized linear mixed models (GLMMs) to further describe predictors of egg rejection. In order to be conservative in the analysis and interpretation of our data, we first controlled for individual females' known propensity to consistently reject or accept foreign eggs irrespective of egg coloration (Croston and Hauber, 2014b; supplementary material Table S2). Although only one nest site was significant (red nest, all egg colors accepted;  $P=0.04$ ) in the model, we also removed a second site that approached significance (robin-mimetic nest, all egg colors rejected;  $P=0.06$ ) to be conservative, thereby excluding two sites at which female robins responded to neither egg color nor nest lining treatment. We therefore excluded a total of six experiments at these two nests from further behavioral analysis, leaving  $N=88$  experiments analyzed in subsequent models (supplementary material Table S2B; Table 2).

We then combined our dataset with published egg rejection data of artificial eggs in natural, non-manipulated robin nests (Croston and Hauber, 2014a). The full model significantly predicted artificial egg rejection/acceptance outcome ( $\chi^2_8=41.19$ ,  $P<0.0001$ ). The only significant predictor of egg rejection in this model was egg color ( $\chi^2_2=39.77$ ,  $P<0.0001$ ; Table 2A). Last, we fitted a GLMM including all above predictors, as well as chromatic and achromatic contrast between egg and nest-lining colors. Again, the whole model significantly predicted egg acceptance/rejection behavior ( $\chi^2_{10}=42.82$ ,  $P<0.0001$ ), but neither chromatic nor achromatic egg–nest JNDs were a significant predictor of egg rejection. As in our above models, the only significant predictor of egg rejection was egg color ( $\chi^2_2=34.05$ ,  $P<0.0001$ ; Table 2B).

To further confirm these results, we ran *post hoc* tests on the single significant predictor (egg color) in the final GLMM model (Table 2B). The *post hoc*  $\chi^2$  test of egg color against a reject/accept outcome variable showed a significant difference in egg rejection behavior by egg color ( $\chi^2_2=40.39$ ,  $P<0.0001$ ; Fig. 5C). Irrespective of nest type, beige eggs were rejected in 33 out of 39 trials, robin-mimetic eggs were rejected in five out of 35 trials, and red eggs were rejected in 23 out of 43 trials. When split by nest type (Fig. 5C,



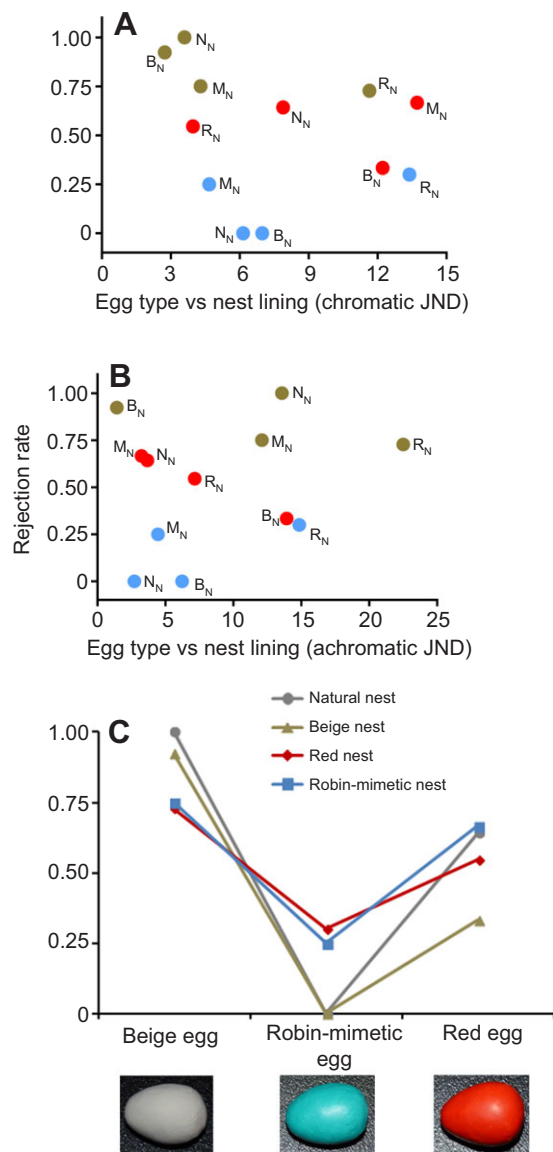
**Fig. 4. Mean (+s.e.m.) chromatic and achromatic contrasts between experimental eggs and all nest linings.** (A,C,E) Chromatic contrasts; (B,D,F) achromatic contrasts. Images below each column indicate the experimental egg–nest lining pair measured (from left to right: natural nest, red nest, robin-mimetic nest, beige nest). All comparisons were made using Kruskal–Wallis rank sums tests followed by Wilcoxon pairwise comparisons. Significant pairwise comparisons are indicated by lowercase letters – columns bearing the same letter are not significantly different. In A and B, beige egg–nest lining chromatic ( $H_3=44.63$ ,  $P<0.0001$ ) and achromatic ( $H_3=43.54$ ,  $P<0.0001$ ) contrasts were significant. In C and D, robin-mimetic egg–nest lining chromatic ( $H_3=42.67$ ,  $P<0.0001$ ) and achromatic ( $H_3=38.03$ ,  $P<0.0001$ ) contrasts were significant. In E and F, red egg–nest lining chromatic ( $H_3=45.00$ ,  $P<0.0001$ ) and achromatic ( $H_3=41.38$ ,  $P<0.0001$ ) contrasts were significant.

Table 1), there was a significant difference in rejection rate of each egg type in beige nests ( $\chi^2_2=24.69$ ,  $P<0.0001$ ). In beige nests, beige eggs were rejected in 12 out of 13 trials, robin-mimetic eggs were rejected in 0 out of nine trials and red eggs were rejected in four out of 12 trials. Further analysis showed that beige eggs were rejected significantly more often than both robin-mimetic eggs ( $\chi^2_1=23.27$ ,  $P<0.001$ ) and red eggs ( $\chi^2_1=10.34$ ,  $P=0.0013$ ; Table 1). Red eggs were similarly rejected more often than robin-mimetic eggs ( $\chi^2_1=5.17$ ,  $P=0.02$ ; Table 1). There was no significant difference in egg rejection by egg color in robin-mimetic nests ( $\chi^2_2=4.68$ ,  $P=0.10$ ; Table 1). In robin-mimetic nests, beige eggs were rejected in six out of eight trials, robin-mimetic eggs were rejected in two out of eight trials and red eggs were rejected in four out of six trials. There was also no significant difference in egg rejection by egg color in red

nests ( $\chi^2_2=3.97$ ,  $P=0.14$ ; Table 1). In red nests, beige eggs were rejected in eight out of 11 trials, robin-mimetic eggs were rejected in three out of 10 trials and red eggs were rejected in six out of 11 trials (Table 1).

## DISCUSSION

Natural nest linings represent a reliable cue against which robins could compare own versus foreign eggs; natural robin nests have low variation in raw reflectance spectra (supplementary material Fig. S1) and avian-perceived chromatic and achromatic visual contrasts across different nests (supplementary material Fig. S2). Furthermore, egg–egg contrasts between natural and artificial egg colors are positively related to egg–nest contrasts in robin nests, thus potentially confounding the interpretation of host–parasite egg



**Fig. 5. The effect of model egg and nest lining color manipulations on egg rejection rates by American robins.** (A,B) Data points refer to egg colors (beige, robin-mimetic (blue) and red) and text refers to nest linings ( $B_N$ , beige nest;  $M_N$ , robin-mimetic nest; and  $R_N$ , red nest). (A) The relationship between chromatic JND of eggs–nest linings and rejection rate was not significant ( $F_{1,10}=0.49$ ,  $P=0.50$ ). (B) The relationship between achromatic JND of eggs–nest linings and rejection rate was also not significant ( $F_{1,10}=0.43$ ,  $P=0.53$ ). (C) Egg ejection rates plotted by nest and egg type, which showed a significant effect of egg type on rejection rate, irrespective of nest treatment (Friedman ANOVA  $\chi^2_2=8.00$ ,  $P=0.018$ ). Egg color was also the only significant predictor of egg rejection in our generalized linear mixed model (GLMM) analysis ( $\chi^2_2=34.05$ ,  $P<0.0001$ ), while nest color was not ( $\chi^2_3=1.84$ ,  $P=0.61$ ). *Post hoc* chi-square tests showed that rejection behavior was consistently ordered by egg colors ( $\chi^2_2=40.39$ ,  $P<0.0001$ ).

rejection studies focusing on egg–egg contrasts only. Yet, our experimental manipulations of nest lining did not reliably alter egg rejection rates. Although we successfully altered the degree of egg–nest visual contrast both above and below natural levels (Table 1, Fig. 4; supplementary material Figs S3 and S5), we show here that the degree of perceivable color difference between foreign eggs and the nest background does not induce a predictable change in rejection rates of foreign eggs in the American robin. We

**Table 2. GLMM fits with binomial distribution of parameters used to assess variables predicting egg rejection behavior**

Predictor	d.f.	$\chi^2$	P-value
<b>A</b>			
Whole model	8	41.19	<0.0001*
Egg color	2	39.77	<0.0001*
Nest color	3	1.13	0.77
Clutch size	1	0.35	0.55
Presentation order	1	0.00	1.00
Experiment date	1	0.03	0.87
<b>B</b>			
Whole model	10	42.82	<0.0001*
Egg color	2	34.05	<0.0001*
Nest color	3	1.84	0.61
Chromatic JND	1	0.31	0.58
Achromatic JND	1	1.51	0.22
Clutch size	1	0.19	0.67
Presentation order	1	0.00	1.00
Experiment date	1	0.13	0.72

GLLM, generalized linear mixed model; outcome variable: accept/reject. JND, just noticeable difference.

Egg rejection data in natural nests sourced from Croston and Hauber (2014a). In A, experimental nests from supplementary material Table S2B and sites at which only one trial was conducted were included (excluding significant sites from supplementary material Table S2A).

In B, all trials included in A were run with the addition of achromatic and chromatic egg–nest lining contrasts as predictor variables (excluding significant sites from supplementary material Table S2A).

Significant models and predictor variables are denoted by an asterisk.

minimally predicted egg–nest contrast would affect rejection rate of red eggs, which are rejected at intermediate rates in natural nests (Fig. 5C; Croston and Hauber, 2014a). Here, red eggs were rejected at intermediate rates irrespective of nest-lining color. Similarly, ejection rates of beige eggs and robin-mimetic eggs remained high and low, respectively, in all experimental nest-lining color conditions (Fig. 5C).

All same-color egg–nest combinations produced the lowest chromatic contrast (i.e. were the most cryptic) when compared with other nest types (e.g. beige egg–beige nest), while different egg–nest combinations consistently yielded high chromatic contrasts (Fig. 4A,C,E). However, the degree of egg–nest chromatic contrast did not have a significant effect on rejection rates in our linear regression analysis (Fig. 5A), and remained non-significant in our GLMM analysis (Table 2B). There was similarly no discernible pattern, nor significant predictive effect, of achromatic contrast on egg rejection (Fig. 4B,D,F, Fig. 5B,C, Table 2). Because only four natural robin eggs went missing throughout the course of this study, excluding the predation of the entire nest (see Materials and methods), we conclude that rejection responses by robins were specifically directed at experimental egg colors, and that manipulation of the nest lining did not induce rejection of the robins' own eggs.

Based on the consistent patterns of relative egg rejection rates between different artificial colors, irrespective of nest type (Fig. 5C, Table 2), we are therefore confident in rejecting the hypothesis that altering egg–nest contrast affects egg rejection in American robins. Unfortunately, robin identity, breeding age, prior experience with natural cowbird parasitism and/or prior experience with our own experimentation were unknown in this study. We also did not collect data on whether robins were flushed during experimental parasitism events, a factor which is known to affect egg rejection behavior in the congeneric European blackbird (*T. merula*; Hanley et al., 2015). Though age and experience may

also influence egg rejection decisions in other brood–parasite host systems, with more experienced individuals typically more likely to correctly identify and reject parasitic eggs (e.g. Moskát et al., 2014a), it is not clear to what extent experience influences rejection decisions in American robins in our study population.

Evidence for parasitic egg crypsis via egg–nest color matching in other brood parasite systems is increasingly well documented in enclosed-nesting species. For example, some bronze-cuckoos (*Chalcites* spp.) have evolved dark egg pigmentation, which is cryptic in the domed nests of their hosts (Langmore et al., 2009). Manipulations could next establish whether host species and/or competing parasites respond differentially to parasitic eggs (Gloag et al., 2014) when experimentally illuminating the nest interior (Cassey, 2009; Honza et al., 2014) or when altering egg–nest contrasts independent of egg–egg contrast (this study). Whether cowbird eggs have a cryptic function in host nests has also not been studied in detail across different *Molothrus* cowbird–host systems (but see Mason and Rothstein, 1987; Siefferman, 2006). For example, cowbird eggs may be cryptic or difficult to see in the open cup nests of the eastern phoebe (*Sayornis phoebe*), which are often built under eaves/bridges or in caves and may be less illuminated than the open cups of robin nests; in turn, phoebes always accept cowbird parasitism (Hauber, 2003a; Peer and Sealy, 2004). Conversely, cowbird eggs have a greater avian-perceivable chromatic contrast against natural robin nest linings than do robin eggs themselves (Fig. 1A), making it unlikely that cowbird eggs are at all cryptic in robin nests.

That the rejection of foreign eggs does not depend on the degree of contrast between eggs and the nest lining (this study) provides support for earlier findings in hosts of egg–mimetic brood parasites that egg rejection is driven mechanistically by differences between foreign and own eggs (Cassey et al., 2008; Stevens et al., 2013; Moskat et al., 2014b). In contrast, cowbird eggs in robin nests are exceptional to this pattern: Croston and Hauber (2014a) showed that while robins' responses to artificial egg colors are generally predicted by chromatic JNDs differentiating foreign versus host eggs, artificial cowbird ground color-mimetic (beige) eggs are rejected in 100% of trials, despite their relatively low avian-perceivable chromatic difference from robin eggs (Fig. 1B). Our experimental manipulations showed that neither chromatic nor achromatic contrasts differentiating foreign eggs from nest linings were significant predictors of egg rejection – thus, cowbird egg rejection is likely the result of comparison between host and foreign eggs in robins (Croston and Hauber, 2014a). Future work should investigate the role of egg–nest contrast in egg rejection using ordinarily non-ejecting hosts.

We should note that higher chromatic contrasts do not necessarily correspond to more robust behavioral responses (Ham and Osorio, 2007). For example, chromatic JNDs differentiating artificial parasitic eggs and natural robin eggs do seem to drive rejection in robins. Specifically, cowbird-mimetic model eggs are rejected at the highest rates despite having relatively low chromatic contrast from robin eggs (Croston and Hauber, 2014a). In the present study, the two visual models showed similar patterns of chromatic contrasts between eggs and nest linings, and our supplementary analyses of physical distance using chromatic PCs largely confirm the outputs of both of our visual models. Despite the corroboration of our visual contrast analyses, we cannot assume that higher JND values in the supra-threshold range necessarily correlate with stronger behavioral responses.

Another caveat in this, and other studies based on the analysis of avian visual modeling data is that the magnitude of the chromatic

difference (whether between eggs or between eggs and nests) is not always a linear means of predicting egg rejection (or any vision-dependent) behavior. Chromatic distance is but one component of broader sensory/perceptual (de la Colina et al., 2012) and cognition-dependent (Hauber and Sherman, 2001; Moskát and Hauber, 2007; Croston and Hauber, 2015) processes that ultimately result in the complex behavioral decision to accept or reject a parasitic egg. For example, there are a growing number of studies showing that perceptual difference alone does not fully explain patterns of egg rejection behavior (Moskát and Hauber, 2007; Moskát et al., 2010; Cassey et al., 2008; Stoddard and Stevens, 2011; Bán et al., 2013; Stevens et al., 2013; Croston and Hauber, 2014a).

Aside from specific perceptual/cognitive processes mediating egg rejection behavior, variation in the predictive power of avian visual models may be partly due to the physiological assumptions made within visual sensory models themselves. Specifically, visual models are based on a limited subset of bird species, including a handful of UVS oscines, none of which are common hosts of brood parasites (Grim et al., 2011; Aidala et al., 2012). Specifically, for this study we used parameters for the robin's visual system from the congeneric European blackbird (*Turdus merula*). This potentially confounds the degree to which we can model and understand host–parasite co-evolution to shape hosts' perceptual sensitivities. It is possible, then, that the visual models used in this and in previous studies do not accurately represent the sensory physiology of the American robin. Likewise, inter-individual differences in sensory physiology could confound our results, such that egg rejection reflects unaccounted-for differences in individual sensory physiology rather than at the level of decision making. Accordingly, within-species differences in sensory physiology have recently been described in the brown-headed cowbird (Fernández-Juricic et al., 2013). Future studies should endeavor not only to obtain and incorporate species-specific models of avian sensory physiology but also to describe the degree of inter-individual variation at both the behavioral and physiological levels.

We have shown here that egg–nest contrast is not a significant predictor of egg rejection by the American robin. Instead, egg rejection in robins is statistically explained, and likely perceptually driven, by differences between the hosts' own eggs and foreign egg colors. Future work should focus on improving visual models by incorporating physiologically appropriate, individual-specific cone densities/absorbance spectra, as well as nest site-specific egg, nest lining and ambient light availability data.

## MATERIALS AND METHODS

### Behavioral experiments

All behavioral experiments were conducted in the vicinity of Ithaca, Tompkins County, NY, USA, from May to July of the 2013 breeding season. We located active robin nests ( $N=48$ ), as defined by dry nest content, warm eggs and/or defense or attendance by adult robins, through focusing on suitable nest sites near human-built structures, as this species is highly commensal (Sallabanks and James, 1999). Nest sites were also located with the help of local citizens via advertising in community Listserv and businesses, and returning to locations with known robin nests from previous years (Croston and Hauber, 2014a,b).

After an active nest containing eggs was located, it was assigned in a balanced random procedure to an experimental nest type (one treatment per nest) and sequential egg treatments (one to three artificial eggs per nest). Robin nests were assigned one of three artificially colored nest linings, and paired with an artificial egg of one of the same three colors (see below for artificial egg and nest details; Fig. 2). Painted felt nest linings (see below for details) were inserted and affixed to the inner bottom lining of robin nests using fast-drying, non-toxic glue (Liquid Fusion<sup>®</sup>). An experimental egg

was then added to the clutch without replacement (removal of one host egg), following methods used by Briskie et al. (1992) for American robins. Although egg replacement by cowbirds has been documented in one-third of parasitized yellow warbler (*Setophaga petechia*) nests (Sealy, 1992) and in most parasitized eastern phoebe (*S. phoebe*) nests (Hauber, 2003a), the addition of an experimental egg does not affect rejection rates in related European *Turdus* thrushes (Davies and Brooke, 1989; Grim et al., 2011) and allowed us to compare our new data with previous studies on robins (Rothstein, 1982; Briskie et al., 1992; Croston and Hauber, 2014a). Following the initiation of an experiment, we remained within sight of the nest to ensure that the new nest lining was not removed by adults upon their return to the nest. Nest lining removal occurred in only 3% of trials, and we returned and replaced the lining. If the experimental nest lining was removed by an adult robin three consecutive times, the experiment was abandoned at that nest. This occurred at only one nest site throughout the entire study.

All nests were checked daily after each experiment was initiated. Eggs were considered rejected if they were missing from a nest upon the return visit, unless the entire clutch was missing (presumed predation) or nestlings had begun to hatch (to avoid conflating egg rejection with eggshell removal, as in nest sanitation: Hauber, 2003c). If an artificial egg remained in the nest on the 5th day after addition, it was considered accepted (Rothstein, 1975; Briskie et al., 1992). In a previous study using the same focal robin population, all ejected model eggs were rejected within 1–4 days of being parasitized (mean 1.69 days; Croston and Hauber, 2014a), justifying a 5 day acceptance threshold. If a model egg remained in the nest through to hatching, we continued monitoring for up to 3 days post-hatching because of well-documented asynchronous hatching in robin broods (Sallabanks and James, 1999; Z.A. personal observations). Following the acceptance or rejection of a first experimental egg, a second egg of a different color was introduced. Up to three different eggs were introduced into robin nests in this way during the laying and incubation periods. The same egg color was not introduced repeatedly into the same nest. The experimental protocols followed in this study were approved by the Hunter College Institutional Animal Care and Use Committee, and all experiments conducted on private properties were done so with the express permission and mostly enthusiastic support from the landowners (Hauber, 2003a; Wagner et al., 2013).

### Experimental eggs and nest linings

We constructed model cowbird eggs within the natural variation of natural brown-headed cowbird egg shape, size (21×16 mm) and mass (2.6–3.4 g) as documented near our field site in upstate New York, USA (Lowther, 1993; Croston and Hauber, 2014a,b; Z.A. personal observation). Model eggs were made from plaster of Paris, using the same silicone molds that were used by Croston and Hauber (2014a,b). Experimental nest-lining inserts were circular discs cut from white felt to fit the bottom of the robin's nest cup dimensions (mean disc diameter 94 mm) at our study site (Fig. 2). Eggs and felt were then painted red, natural cowbird ground color-mimetic (beige) or blue–green (robin-mimetic), using the same latex or acrylic paint as used in Croston and Hauber (2014a). We utilized the three egg and nest lining colors by considering the general shape and peak of their reflectance curves and by the relative photon catches of each avian cone photoreceptor (Endler, 1990; Endler and Mielke, 2005; Fig. 3), predicted to induce sharply different sensory responses of the UV-sensitive visual range of American robins (Aidala et al., 2012). We also chose these three egg/nest colors because they represented known behavioral variation in egg ejection responses in natural nests within the same population of robins: beige (100% rejected), red (64% rejected) and robin-mimetic (0% rejected; Croston and Hauber, 2014a). These extreme and intermediate egg color rejection rates allowed us to design a two-tailed experiment, whereby both increased, decreased and unchanged rejection rates would be predicted as a result of our experimental manipulations (Table 1).

As an internal experimental control for our invasive manipulations, we monitored the fate of naturally laid robin eggs in each clutch: a total of four robin eggs (at  $N=48$  nests monitored, mean natural clutch size per nest=3.3 eggs) went missing during our study in 2013 (outside of complete nest predation events), implying that egg rejection responses were limited to experimental model eggs, and that own-egg rejection was not related to experimental manipulation of the nest lining. It was unclear in these

instances whether these eggs were missing as a result of partial depredation events or failed rejections. In turn, as experimental controls for the nest lining manipulation, we contrasted the data from all of our experiments (single- and multiple-presentation nests in 2013 with the published behavioral egg rejection data in natural, non-manipulated robin nests from Croston and Hauber (2014a), barring those from the two excluded sites in the GLMM model described below and in supplementary material Table S2. We acknowledge the limitation that using the published egg rejection data from natural nests is at best a partial methodological control for our nest lining manipulations, and a full experimental control should conceivably include adding a see-through felt, or felt dyed with a natural nest reflectance matching color. Furthermore, those data were derived mostly during the 3 years prior to our experiments; however, egg rejection rates did not vary between years in our study population (Croston and Hauber, 2014a,b).

### Spectral measurements and visual modeling

We obtained spectral measurements of natural robin ( $N=76$ ) and cowbird ( $N=15$ ) eggs by combining our dataset from 2013 with that of Croston and Hauber (2014a). In 2013, we also collected reflectance spectra from natural robin nest linings ( $N=19$ ), as well as from our artificial eggs and nest backgrounds. Spectral measurements were taken with an Ocean Optics USB2000 Miniature Fiber Optic Spectrometer, connected to a laptop computer running OOIBase32 software, and using a UV-Vis DT mini-lamp light source (Ocean Optics, Inc. Dunedin, FL, USA) or an Ocean Optics Jaz spectrometer with UV-VIS light source (Ocean Optics, Inc.). All measurements were taken at a 90 deg angle to the egg or nest-lining surface. We took nine measurements each from individual nests, linings and eggs: three measurements each from the nests' upper inner cup, lower inner cup and bottom; and three measurements each from the blunt pole, middle portion and narrow pole of natural and artificial eggs (Croston and Hauber, 2015). The spectrometer was re-calibrated frequently, using the Ocean Optics WS-1 white reflectance standard and a dark reference made from a cardboard box, lined with black felt, and pierced to create a small hole for the probe (blocking any incident light; Igic et al., 2009; Igic et al., 2010; Croston and Hauber, 2014a). We averaged the nine spectra per egg/nest to generate a composite spectra profile for each egg and nest included in our visual modeling analyses. As a methodological check, we compared the mean achromatic and chromatic spectra of each nest lining area prior to compiling composite natural nest lining spectra.

Visual modeling analyses were conducted using AVICOL v.6 (Gomez, 2006). We applied a 15 nm triangular correction to raw spectra, available as a function within AVICOL, to attenuate and minimize the effect of spectrometer noise on the visual model. We ran a tetrachromatic receptor noise-limited color opponency model (Vorobyev and Osorio, 1998), assuming noise independent of the neural signal, and set the Weber fraction to 0.1 (Vorobyev et al., 1998; Igic et al., 2010; Croston and Hauber, 2014a,b, 2015). This type of opponency contrast model is preferable over avian visual models only accounting for properties of the photoreceptors themselves because such models do not agree with behavioral psychophysics data (see Vorobyev and Osorio, 1998). The model incorporates maximal absorbance and relative densities of each cone type as well as other physiological variables such as oil droplet and ocular media transmittance, allowing for analysis of both chromatic and achromatic contrasts (Vorobyev and Osorio, 1998; Vorobyev et al., 1998).

Because no photoreceptor absorbance or relative cone density data are currently available for robins, we approximated photoreceptor abundance and relative cone density based on published data of the closely related UVS European blackbird (Hart et al., 2000). The use of a congener *Turdus* may be suitable as the American robin is predicted to also possess a UVS SWS1 photopigment, based on the results of our molecular genetic analyses of the *SWS1* opsin gene of the robin (Aidala et al., 2012). In this model, we set the relative cone densities (UVS: 1, SWS: 1.78, MWS: 2.21, LWS: 1.96) based on cone density data measured by Hart et al. (2000). Ambient light level irradiance data of a generic 'open-cup' nesting species were extracted from Avilés et al. (2008) and were kindly provided by Igic et al. (2012), as ambient light levels can affect both the risk of parasitism and parasitic egg detection (Langmore et al., 2005; Muñoz et al., 2007; Avilés, 2008; Honza et al., 2011).



Achromatic contrasts were calculated by summing MWS and LWS cone spectra (Osorio and Vorobyev, 2005, 2008; Gomez, 2006), as their combined sensitivities are thought to be comparable to those of the non-color-sensitive rod and double cone (Osorio et al., 1999) photoreceptors across avian taxa (Hart et al., 1998, 2000; Igic et al., 2009). Using the model parameters described above, AVICOL generated separate chromatic and achromatic perceptual distances between two objects as JNDs; a calculated JND value greater than 1.0 suggests that two stimuli are discriminable from one another, while a JND less than 1.0 suggests that they are not (Gomez, 2006).

Although our visual modeling is based on the known retinal physiology of a closely related UVS *Turdus* species, the European blackbird, we augmented our visual modeling analyses by also computing a VS visual model as differences in retinal physiology between the European blackbird and the American robin are unknown. In this second model, we used the cone absorbance spectra from the VS rock pigeon (*Columba livia*; Bowmaker et al., 1997; Vorobyev and Osorio, 1998) and the relative cone densities (UVS: 1, SWS: 1.9, MWS: 2.2, LWS: 2.1) of the peafowl (*Pavo cristatus*) as measured by Hart (2002). All other visual modeling parameters remained the same as in our UVS visual model.

We also analyzed raw reflectance spectra of nests and eggs, as reliance on avian visual modeling alone introduces untested assumptions about a focal species' physiology (Stoddard and Stevens, 2011), and can be avoided by analyzing raw spectra instead (Cherry and Bennett, 2001; Starling et al., 2006; Cherry et al., 2007; but see Endler and Mielke, 2005). We conducted principal components analysis (PCA) on covariances of interpolated egg and nest reflectance spectra over 1 nm intervals from 300 to 700 nm. In such analyses, PC1 represents the majority of the variance and is a measure of achromatic/brightness variation, while PC2 and PC3 represent chromatic variation (Cherry and Bennett, 2001; Endler and Mielke, 2005; supplementary material Fig. S4A). In our analysis, the first three PCs explained 98.6% of the variance (supplementary material Table S1; PC1=67.14%, PC2=28.53% and PC3=2.93%). Subsequent PCs explained less than 1% of the variance each and were eliminated from further analysis. We calculated the absolute value distance between PC1 scores for achromatic egg–nest contrasts (following Igic et al., 2012). As a measure of chromatic distance, we calculated the Euclidean distance between principal components scores for PC2 (described by  $x$ - and  $y$ -coordinate values of  $p_1$  and  $p_2$ ) and PC3 (described by  $x$ - and  $y$ -coordinate values of  $q_1$  and  $q_2$ ; supplementary material Fig. S4B) using the standard distance formula:

$$d = \sqrt{(q_1 - p_1)^2 + (q_2 - p_2)^2}. \quad (1)$$

Using these distance scores, we examined the relationship between (a) chromatic distance and rejection rates between experimental eggs and nest linings using linear regression. These additional analyses of our spectral data allowed for increased explanatory power of our behavioral results as they relate to visual contrast, and complemented the statistical and qualitative conclusions drawn from our JND analyses.

### Data analysis

In order to confirm that our natural nest lining composite spectra were representative of all three nest areas measured, and not biased towards one nest area over the others, we compared avian-perceived (a)chromatic differences between natural nest lining areas (upper inner cup, lower inner cup and bottom). No achromatic or chromatic within-nest area comparison was higher than 1.65 JNDs. Because the visual contrasts between nest areas were so low, we used the composite natural nest lining spectra including the nine measurements from the three nest areas in all analyses. In order to show that there exists a methodological confound between egg–egg chromatic contrast and egg–nest chromatic contrast, we conducted non-parametric Mann–Whitney  $U$ -tests between natural robin and cowbird eggs against conspecific natural robin eggs and natural robin nest linings, respectively. We also conducted a linear regression analysis to test the relationship between egg–natural robin egg and egg–natural robin nest lining chromatic contrasts using artificial egg stimuli sourced from Croston and Hauber (2014a). We next confirmed that our nest lining manipulations resulted in

experimental alteration of chromatic and achromatic contrasts between eggs and nests, using non-parametric Kruskal–Wallis rank sums tests and *post hoc* pairwise comparisons following the Wilcoxon method. Prior to analysis, we randomized our comparisons such that only one egg–nest combination was used in each type of egg–nest contrast comparison.

We examined the statistical relationship between (a)chromatic egg–nest and egg–egg contrasts and rejection rates for both natural and artificial eggs using linear regression analyses. A non-parametric 2-way Friedman ANOVA was run to test whether nest color affected egg rejection behavior across the different egg color stimuli. To further examine the role of egg–egg and egg–nest contrasts in parasitic egg rejections, we fitted binomial GLMMs (with accept/reject as the outcome variables) using Firth-adjusted bias estimates to determine the degree to which nest color influenced egg rejection behavior. In these models, we included egg color, nest color, nest site, experimental date, presentation order and natural clutch size as predictor variables (Table 2; supplementary material Table S2). We first examined the known effects of the same individual female robins' tendencies to consistently accept or reject differently colored eggs (Croston and Hauber, 2014b) across nest treatments by nesting site within nest color (supplementary material Table S2). In these models, we only included nest sites where more than one egg had been presented. After controlling for individual females' tendencies to accept or reject experimental eggs irrespective of egg/nest treatments (supplementary material Table S2), we included rejection rates in natural nests (Croston and Hauber, 2014a) for the three egg colors used in this study. We included the same predictors listed above except nest site. Lastly, we included chromatic and achromatic JNDs between eggs and nests in the GLMM model to explicitly test the role of avian-perceived contrasts in egg rejection frequencies. *Post hoc* analyses of significant predictors in this final GLMM were run using chi-square tests. All analyses were run using JMP v. 10 (SAS Institute, Inc., Cary, NC, USA), Statview 5.1 (SAS Institute, Inc.), and GraphPad Prism v. 6 (GraphPad Software, Inc., La Jolla, CA, USA). Figures were compiled and edited using Adobe Creative Suite 5 (Adobe Systems, Inc., San Jose, CA, USA).

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Z.A. and M.E.H. designed this study. Z.A., R.C., J.S. and L.T. conducted the experiments and collected data. Z.A. and M.E.H. analyzed the data and Z.A. wrote the first draft of the manuscript, with all authors contributing to critical interpretation of data and results, and the writing and revision of subsequent drafts of the manuscript.

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### Supplementary material

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### References

- Abernathy, V. E. and Peer, B. D. (2014). Intraclutch variation in egg appearance of brown-headed cowbird hosts. *Auk* **131**, 467–475.
- Aidala, Z., Huynen, L., Brennan, P. L. R., Musser, J., Fidler, A., Chong, N., Machovsky Capuska, G. E., Anderson, M. G., Talaba, A., Lambert, D. et al. (2012). Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines. *J. Comp. Physiol. A* **198**, 495–510.

- Avilés, J. M.** (2008). Egg colour mimicry in the common cuckoo *Cuculus canorus* as revealed by modelling host retinal function. *Proc. R. Soc. Lond. B Biol. Sci.* **275**, 2345–2352.
- Avilés, J. M., Soler, J. J., Pérez-Contreras, T., Soler, M. and Møller, A. P.** (2005). Ultraviolet reflectance of great spotted cuckoo eggs and egg discrimination by magpies. *Behav. Ecol.* **17**, 310–314.
- Avilés, J. M., Pérez-Contreras, T., Navarro, C. and Soler, J. J.** (2008). Dark nests and conspicuousness in color patterns of nestlings of altricial birds. *Am. Nat.* **171**, 327–338.
- Avilés, J. M., Vikan, J. R., Fossøy, F., Antonov, A., Moksnes, A., Røskaft, E. and Stokke, B. G.** (2010). Avian colour perception predicts behavioural responses to experimental brood parasitism in chaffinches. *J. Evol. Biol.* **23**, 293–301.
- Bailey, I. E., Muth, F., Morgan, K., Meddle, S. L. and Healy, S. D.** (2015). Birds build camouflaged nests. *Auk* **132**, 11–15.
- Bán, M., Moskát, C., Barta, Z. and Hauber, M. E.** (2013). Simultaneous viewing of own and parasitic eggs is not required for egg rejection by a cuckoo host. *Behav. Ecol.* **24**, 1014–1021.
- Bowmaker, J. K., Heath, L. A., Wilkie, S. E. and Hunt, D. M.** (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res.* **37**, 2183–2194.
- Briskie, J. V., Sealy, S. G. and Hobson, K. A.** (1992). Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution* **46**, 334–340.
- Cassey, P.** (2009). Biological optics: seeing colours in the dark. *Curr. Biol.* **19**, R1083–R1084.
- Cassey, P., Honza, M., Grim, T. and Hauber, M. E.** (2008). The modelling of avian visual perception predicts behavioural rejection responses to foreign egg colours. *Biol. Lett.* **4**, 515–517.
- Cherry, M. I. and Bennett, A. T. D.** (2001). Egg colour matching in an African cuckoo, as revealed by ultraviolet-visible reflectance spectrophotometry. *Proc. R. Soc. Lond. B Biol. Sci.* **268**, 565–571.
- Cherry, M. I., Bennett, A. T. D. and Moskát, C.** (2007). Host intra-clutch variation, cuckoo egg matching and egg rejection by great reed warblers. *Naturwissenschaften* **94**, 441–447.
- Croston, R. and Hauber, M. E.** (2014a). Spectral tuning and perceptual differences do not explain the rejection of brood parasitic eggs by American robins (*Turdus migratorius*). *Behav. Ecol. Sociobiol.* **68**, 351–362.
- Croston, R. and Hauber, M. E.** (2014b). High repeatability of egg rejection in response to experimental brood parasitism in the American robin (*Turdus migratorius*). *Behaviour* **151**, 703–718.
- Croston, R. and Hauber, M. E.** (2015). Experimental changes in intraclutch egg color variation do not covary with egg rejection in a host of a non-egg-mimetic avian brood parasite. *PLoS ONE* (in press).
- Davies, N. B.** (2000). *Cuckoos, Cowbirds, and Other Cheats*. London: Poyser.
- Davies, N. B.** (2011). Cuckoo adaptations: trickery and tuning. *J. Zool.* **284**, 1–14.
- Davies, N. B. and Brooke, M. D. L.** (1989). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *J. Anim. Ecol.* **58**, 207–224.
- Davies, N. B., Brooke, M. D. L. and Kacelnik, A.** (1996). Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 925–931.
- de la Colina, M. A., Pompilio, L., Hauber, M. E., Reboreda, J. C. and Mahler, B.** (2012). Different recognition cues reveal the decision rules used for egg rejection by hosts of a variably mimetic avian brood parasite. *Anim. Cogn.* **15**, 881–889.
- Endler, J. A.** (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.
- Endler, J. A. and Mielke, P. W.** (2005). Comparing entire colour patterns as birds see them. *Biol. J. Linn. Soc.* **86**, 405–431.
- Fernández-Juricic, E., Ojeda, A., Deisher, M., Burry, B., Baumhardt, P., Stark, A., Elmore, A. G. and Ensminger, A. L.** (2013). Do male and female cowbirds see their world differently? Implications for sex differences in the sensory system of an avian brood parasite. *PLoS ONE* **8**, e58985.
- Gloag, R., Keller, L.-A. and Langmore, N. E.** (2014). Cryptic cuckoo eggs hide from competing cuckoos. *Proc. R. Soc. Lond. B Biol. Sci.* **281**, 20141014.
- Gomez, D.** (2006). AVICOL, a program to analyse spectrometric data. Last update October 2011. Free executable available at <http://sites.google.com/site/avicolprogram/> or from the author at [dodogomez@yahoo.fr](mailto:dodogomez@yahoo.fr).
- Grim, T., Samaš, P., Moskát, C., Kleven, O., Honza, M., Moksnes, A., Røskaft, E. and Stokke, B. G.** (2011). Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? *J. Anim. Ecol.* **80**, 508–518.
- Guigueno, M. F. and Sealy, S. G.** (2012). Nest sanitation in passerine birds: implications for egg rejection in hosts of brood parasites. *J. Ornithol.* **153**, 35–52.
- Ham, A. D. and Osorio, D.** (2007). Colour preferences and colour vision in poultry chicks. *Proc. R. Soc. Lond. B Biol. Sci.* **274**, 1941–1948.
- Hanley, D., Samaš, P., Heryán, J., Hauber, M. E. and Grim, T.** (2015). Now you see it, now you don't: flushing hosts prior to experimentation can predict their responses to brood parasitism. *Sci. Rep.* **5**, 9060.
- Hart, N. S.** (2002). Vision in the peafowl (*Aves: Pavo cristatus*). *J. Exp. Biol.* **205**, 3925–3935.
- Hart, N. S., Partridge, J. and Cuthill, I.** (1998). Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (*Sturnus vulgaris*). *J. Exp. Biol.* **201**, 1433–1446.
- Hart, N. S., Partridge, J. C., Cuthill, I. C. and Bennett, A. T. D.** (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **186**, 375–387.
- Hauber, M. E.** (2003a). Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. *Behav. Ecol.* **14**, 227–235.
- Hauber, M. E.** (2003b). Interspecific brood parasitism and the evolution of host clutch sizes. *Evol. Ecol. Res.* **5**, 559–570.
- Hauber, M. E.** (2003c). Egg-capping is a cost paid by hosts of interspecific brood parasites. *Auk* **120**, 860–865.
- Hauber, M. E. and Sherman, P. W.** (2001). Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* **24**, 609–616.
- Hauber, M. E., Moskát, C. and Bán, M.** (2006). Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biol. Lett.* **2**, 177–180.
- Honza, M. and Polačiková, L.** (2008). Experimental reduction of ultraviolet wavelengths reflected from parasitic eggs affects rejection behaviour in the blackcap *Sylvia atricapilla*. *J. Exp. Biol.* **211**, 2519–2523.
- Honza, M., Polačiková, L. and Procházka, P.** (2007). Ultraviolet and green parts of the colour spectrum affect egg rejection in the song thrush (*Turdus philomelos*). *Biol. J. Linn. Soc.* **92**, 269–276.
- Honza, M., Procházka, P., Morongová, K., Čapek, M. and Jelínek, V.** (2011). Do nest light conditions affect rejection of parasitic eggs? A test of the light environment hypothesis. *Ethology* **117**, 539–546.
- Honza, M., Šulc, M. and Cherry, M. I.** (2014). Does nest luminosity play a role in recognition of parasitic eggs in domed nests? A case study of the red bishop. *Naturwissenschaften* **101**, 1009–1015.
- Hoover, J. P.** (2003). Multiple effects of brood parasitism reduce the reproductive success of prothonotary warblers, *Protonotaria citrea*. *Anim. Behav.* **65**, 923–934.
- Igic, B., Grim, T. and Hauber, M. E.** (2009). Cigarette butts form a perceptually cryptic component of song thrush (*Turdus philomelos*) nests. *Notornis* **56**, 134–138.
- Igic, B., Leuschner, N., Parker, K. A., Ismar, S. M. H., Gill, B. J., Lovegrove, T. G., Millar, C. D. and Hauber, M. E.** (2010). Size dimorphism and avian-perceived sexual dichromatism in a New Zealand endemic bird, the whitehead *Mohoua albigilla*. *J. Morphol.* **271**, 697–704.
- Igic, B., Cassey, P., Grim, T., Greenwood, D. R., Moskát, C., Rutila, J. and Hauber, M. E.** (2012). A shared chemical basis of avian host-parasite egg colour mimicry. *Proc. R. Soc. Lond. B Biol. Sci.* **279**, 1068–1076.
- Kilner, R. M. and Langmore, N. E.** (2011). Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biol. Rev.* **86**, 836–852.
- Lahti, D. C.** (2006). Persistence of egg recognition in the absence of cuckoo brood parasitism: pattern and mechanism. *Evolution* **60**, 157–168.
- Lahti, D. C. and Lahti, A. R.** (2002). How precise is egg discrimination in weaverbirds? *Anim. Behav.* **63**, 1135–1142.
- Langmore, N. E., Kilner, R. M., Butcher, S. H. M., Maurer, G., Davies, N. B., Cockburn, A., Macgregor, N. A., Peters, A., Magrath, M. J. L. and Dowling, D. K.** (2005). The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behav. Ecol.* **16**, 686–692.
- Langmore, N. E., Stevens, M., Maurer, G. and Kilner, R. M.** (2009). Are dark cuckoo eggs cryptic in host nests? *Anim. Behav.* **78**, 461–468.
- Lawes, M. J. and Kirkman, S.** (1996). Egg recognition and interspecific brood parasitism rates in red bishops (*Aves: Ploceidae*). *Anim. Behav.* **52**, 553–563.
- López-de-Hierro, M. D. G. and Moreno-Rueda, G.** (2010). Egg-spot pattern rather than egg colour affects conspecific egg rejection in the house sparrow (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **64**, 317–324.
- Lorenzana, J. C. and Sealy, S. G.** (2001). Fitness costs and benefits of cowbird egg ejection by gray catbirds. *Behav. Ecol.* **12**, 325–329.
- Lowther, P. E.** (1993). Brown-headed cowbird (*Molothrus ater*). In *The Birds of North America Online* (ed. A. Poole). Ithaca: Cornell Laboratory of Ornithology. Retrieved from The Birds of North America Online database: <http://bna.birds.cornell.edu>.
- Marchetti, K.** (2000). Egg rejection in a passerine bird: size does matter. *Anim. Behav.* **59**, 877–883.
- Mason, P. and Rothstein, S. I.** (1987). Crypsis versus mimicry and the color of shiny cowbird eggs. *Am. Nat.* **130**, 161–167.
- Moskát, C. and Hauber, M. E.** (2007). Conflict between egg recognition and egg rejection decisions in common cuckoo (*Cuculus canorus*) hosts. *Anim. Cogn.* **10**, 377–386.
- Moskát, C., Székely, T., Cuthill, I. C. and Kisbenedek, T.** (2008). Hosts' responses to parasitic eggs: which cues elicit hosts' egg discrimination? *Ethology* **114**, 186–194.
- Moskát, C., Bán, M., Székely, T., Komdeur, J., Lucassen, R. W. G., van Boheemen, L. A. and Hauber, M. E.** (2010). Discordancy or template-based recognition? Dissecting the cognitive basis of the rejection of foreign eggs in hosts of avian brood parasites. *J. Exp. Biol.* **213**, 1976–1983.

- Moskát, C., Bán, M. and Hauber, M. E.** (2014a). Naïve hosts of avian brood parasites accept foreign eggs, whereas older hosts fine-tune foreign egg discrimination during laying. *Front. Zool.* **11**, 45.
- Moskát, C., Zölei, A., Bán, M., Elek, Z., Tong, L., Geltsch, N. and Hauber, M. E.** (2014b). How to spot a stranger's egg? A mimicry-specific discordancy effect in the recognition of parasitic eggs. *Ethology* **120**, 616-626.
- Muñoz, A. R., Altamirano, M., Takasu, F. and Nakamura, H.** (2007). Nest light environment and the potential risk of common cuckoo (*Cuculus canorus*) parasitism. *Auk* **124**, 619-627.
- Øien, I. J., Moksnes, A., Røskaft, E. and Honza, M.** (1998). Costs of cuckoo *Cuculus canorus* parasitism to reed warblers *Acrocephalus scirpaceus*. *J. Avian Biol.* **29**, 209-215.
- Osorio, D. and Vorobyev, M.** (2005). Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. Lond. B Biol. Sci.* **272**, 1745-1752.
- Osorio, D. and Vorobyev, M.** (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Res.* **48**, 2042-2051.
- Osorio, D., Miklósi, A. and Gonda, Z.** (1999). Visual ecology and perception of coloration patterns by domestic chicks. *Evol. Ecol.* **13**, 673-689.
- Peer, B. D. and Sealy, S. G.** (2004). Correlates of egg rejection in hosts of the brown-headed cowbird. *Condor* **106**, 580-599.
- Reeve, H. K.** (1989). The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407-435.
- Rodríguez-Gironés, M. A. and Lotem, A.** (1999). How to detect a cuckoo egg: a signal-detection theory model for recognition and learning. *Am. Nat.* **153**, 633-648.
- Rothstein, S. I.** (1975). An experimental and teleonomic investigation of avian brood parasitism. *Condor* **77**, 250-271.
- Rothstein, S. I.** (1982). Mechanisms of avian egg recognition: which egg parameters elicit responses by rejecter species? *Behav. Ecol. Sociobiol.* **11**, 229-239.
- Sallabanks, R. and James, F.** (1999). American robin (*Turdus migratorius*). In *The Birds of North America Online* (ed. A. Poole). Ithaca: Cornell Laboratory of Ornithology. Retrieved from The Birds of North America Online database: <http://bna.birds.cornell.edu/bna/species/462>.
- Sealy, S. G.** (1992). Removal of yellow warbler eggs in association with cowbird parasitism. *Condor* **94**, 40-54.
- Siefferman, L.** (2006). Egg coloration and recognition of conspecific brood parasitism in eastern bluebirds. *Ethology* **112**, 833-838.
- Spottiswoode, C. N. and Stevens, M.** (2010). Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs. *Proc. Natl. Acad. Sci. USA* **107**, 8672-8676.
- Starling, M., Heinsohn, H., Cockburn, A. and Langmore, N. E.** (2006). Cryptic genes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1929-1934.
- Stevens, M., Troscianko, J. and Spottiswoode, C. N.** (2013). Repeated targeting of the same hosts by a brood parasite compromises host egg rejection. *Nat. Commun.* **4**, 2475.
- Stoddard, M. C. and Stevens, M.** (2010). Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. Lond. B Biol. Sci.* **277**, 1387-1393.
- Stoddard, M. C. and Stevens, M.** (2011). Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution* **65**, 2004-2013.
- Stoddard, M. C., Kilner, R. M. and Town, C.** (2014). Pattern recognition algorithm reveals how birds evolve individual egg pattern signatures. *Nat. Commun.* **5**, 4117.
- Thorogood, R. and Davies, N. B.** (2013). Reed warbler hosts fine-tune their defenses to track three decades of cuckoo decline. *Evolution* **67**, 3545-3555.
- Vorobyev, M. and Osorio, D.** (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B Biol. Sci.* **265**, 351-358.
- Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J. and Cuthill, I. C.** (1998). Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **183**, 621-633.
- Wagner, G. F., Aidala, Z., Croston, R. and Hauber, M. E.** (2013). Repeated brood parasitism by brown-headed cowbirds (*Molothrus ater*) at nesting sites of eastern phoebes (*Sayornis phoebe*) across non-consecutive years. *Wilson J. Ornithol.* **125**, 389-394.