

# Does contrast between eggshell ground and spot coloration affect egg rejection?

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**Abstract** Obligate avian brood parasitic species impose the costs of incubating foreign eggs and raising young upon their unrelated hosts. The most common host defence is the rejection of parasitic eggs from the nest. Both egg colours and spot patterns influence egg rejection decisions in many host species, yet no studies have explicitly examined the role of variation in spot coloration. We studied the American robin *Turdus migratorius*, a blue-green unspotted egg-laying host of the brown-headed cowbird *Molothrus ater*, a brood parasite that lays non-mimetic spotted eggs. We examined host responses to model eggs with variable spot coloration against a constant robin-mimetic ground colour to identify patterns of rejection associated with perceived contrast between spot and ground colours. By using avian visual modelling, we found that robins were more likely to reject eggs whose spots had greater chromatic (hue) but not achromatic (brightness) contrast. Therefore, egg rejection decision rules in the American

robin may depend on the colour contrast between parasite eggshell spot and host ground coloration. Our study also suggests that egg recognition in relation to spot coloration, like ground colour recognition, is tuned to the natural variation of avian eggshell spot colours but not to unnatural spot colours.

**Keywords** American robin · Brood parasitism · Chroma · Egg rejection · Spectrometry · Spot coloration

## Introduction

Obligate avian brood parasites lay their eggs into other species' nests, which imposes the cost of parental care upon these foster parents (Davies 2000). Parasitized nests experience reduced reproductive success not only because of misdirected parental care to unrelated offspring, but also due to the parasitic offspring outcompeting or eliminating host offspring (Servedio and Hauber 2006; Moskát et al. 2008). Hosts may reduce or avoid the many costs associated with raising unrelated offspring by evolving defences against brood parasites during the laying, incubation or chick stages (Grim et al. 2011). However, the recognition and rejection of foreign eggs laid in the nest seem to be the most specific, common and adaptive defence that hosts have evolved (Rothstein 1975; Davies 2000).

The success of individual hosts in combating brood parasites depends, in part on their abilities to discriminate these foreign eggs from their own and to make appropriate rejection decisions (Bán et al. 2013). Hosts typically discriminate foreign eggs by visual cues of shape, colour, pattern and/or size (Mason and Rothstein 1986; Underwood and Sealy 2006; Avilés et al. 2010; Spottiswoode and Stevens 2010, 2011; Stevens et al. 2013). Egg recognition studies using foreign real or model eggs, experimentally introduced into host nests,

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provide insights into coevolutionary interactions between avian hosts and their brood parasites (Lyon and Eadie 1991; Rothstein and Robinson 1998; Hauber et al. 2015).

Prior observational and experimental work has focused on the role of eggshell ground coloration and spot (maculation) patterning distribution in eliciting egg rejection responses (Polačiková et al. 2008; López-de-Hierro and Moreno-Rueda 2009). However, it remains uncertain whether hosts employ cognitive rules when rejecting eggs based on the perceived contrast between eggshell ground coloration and spot coloration. Eggshell spots are generated by the deposition of protoporphyrin into or atop the eggshell matrix (Cassey et al. 2010; Deeming and Reynolds 2015) and its variable concentration generates a range of subtractive admixtures with the underlying pigments and calcite matrix can generate a variety of spot colours (Igic et al. 2010, 2012; Hanley et al. 2015a). Many species of birds produce spotted eggs, and the mimicry of these spotting patterns has been studied as a component of a brood parasite's strategy of deception (Swynnerton 1918; Davies and Brooke 1989b; Kilner 2006; Stoddard and Stevens 2010, 2011).

American robins (*Turdus migratorius*; hereafter robins) are occasional hosts of the obligatory brood parasitic brown-headed cowbird *Molothrus ater* (Friedmann et al. 1977; Ortega 1998). Robins accept eggs that are painted a mimetic blue-green colour closely matching the spectral range of real robin eggs (Croston and Hauber 2014b; Aidala et al. 2015; Dinets et al. 2015, Luro and Hauber 2017). By contrast, they consistently reject natural cowbird and artificial eggs that resemble cowbird eggs (Rothstein 1982; Croston and Hauber 2014b; Igic et al. 2015). Importantly, the presence or absence of dark, cowbird-like spots on artificial eggs was a strong predictor of egg rejection by robins when applied against a robin-mimetic ground colour (Rothstein 1982).

Here, we employed perceptual models of avian colour vision (Cassey et al. 2008) to test how variation in the perceivable contrast between spot colours and ground coloration affects hosts' rejection responses. We designed acrylic paint mixtures that varied continuously along the diversity of possible natural eggshell colours as birds would perceive them (Hanley et al. 2015a, 2017). We painted 3D printed model eggs with a blue-green ground colour mimetic of robin eggshell coloration (Igic et al. 2015) but with spots that had colours that varied along the natural diversity of egg coloration (see Hanley et al. 2017). Then, we placed these model eggs into active robin nests and monitored their outcome over 5 days. Each egg's spot colour was unique along this continuous colour continuum rather than being grouped into discrete egg-type categories as was done in majority of previous studies; such artificial categorization might bias results of egg rejection studies (for discussion, see Grim 2005).

Unlike humans, birds possess four photoreceptors sensitive to light ranging from human-invisible ultraviolet wavelengths

to human-visible wavelengths (300 to 700 nm). Accounting for avian vision is therefore crucial and has led to advances in the field of avian perception (Stuart-Fox and Moussalli 2008) and brood parasitism (Stoddard and Stevens 2011, Hanley et al. 2017). The perception of colours can broadly be classified as chromatic aspects, relating to differences in colour, and achromatic aspects, relating to differences in brightness. These two aspects of colour perception are believed to be processed through two independent mechanisms related to the photoreceptors and double cones, respectively (Stoddard and Prum 2011). The chromatic characteristic of eggshell colours has been used to evaluate eggshell mimicry in brood parasites (Stoddard and Stevens 2011). The achromatic aspect of a colour can also influence the way an egg is perceived by a host, thus affecting eggshell discrimination (Spottiswoode and Stevens 2010), particularly in low light conditions (Avilés 2008). Here, we quantify both chromatic and achromatic contrasts between spot and ground colour when evaluating our experimental egg models. We calculated the chromatic and achromatic just noticeable differences (hereafter JNDs) between the spot colours used on our experimental model eggs and the ground colour of these eggs as a measure of internal difference (hereafter experimental comparison). In addition, we calculated the difference between the spot colour and the ground colour of the natural robin eggs (hereafter natural comparison). We then explored if egg rejection response rates by robins were affected by variation in either of these calculated metrics. Thus, in this study, we provide the first test of whether robins respond to perceived contrast of egg spot coloration by egg rejection. Specifically, our aim was to explore when we would begin to see an increased rejection in response spot colour contrast (Hauber et al. 2006; Moskát and Hauber 2007).

## Methods and materials

### Study area and procedure

The fieldwork was conducted in Ithaca, Tompkins County, NY, USA, between May 28 and June 13 of 2015 (for the study site's description and details, see Croston and Hauber 2014b). Robins at this site often nest in orchards, throughout residential gardens, and on human-made structures. Nests were located in an assortment of vegetation types and on or near building exteriors. Nests were considered suitable for artificial parasitism experiments if they were active, located  $\leq 5$  m above ground, contained  $\geq 2$  eggs at the time of discovery and adult robins were observed in or near the nest area (i.e., tree, bush or human-made structure). All data for this study were acquired following approval by the Hunter College Institutional Animal Care and Use Committee (MH 2/16-T3).

Active nests were parasitized at any stage so long as there were no chicks in the nest on the date of parasitism, all nests in

our total data set were parasitized within a 16-day period. While cowbirds predominantly parasitize nests during the host laying stage, the timing of parasitism relative to host nest age does not impact host rejection decision-making in most hosts of brood parasites, including *Turdus* thrushes (Grim et al. 2014). Moreover, previous research on this population has found that date of parasitism did not significantly predict robin's egg rejection responses (Croston and Hauber 2014a). Although we randomly selected experimental eggs ( $n = 44$ ) for each trial, the date of experimental parasitism negatively correlated with the contrast between experimental eggshell colour and spot colour ( $r = -0.50$ ,  $CI_{0.95} = -0.70$  to  $-0.24$ ,  $p < 0.001$ ). Given this information, specific to our population, we excluded data of parasitism from further analysis as it would preclude us from analysing the effect of our experimentally manipulated spot colours on egg rejection events due to multicollinearity (Zuur et al. 2010).

Each experimentally parasitized nest was observed once daily for five consecutive days (Croston and Hauber 2014b). Without touching the eggs, an extendable mirror on a metal pole was used to observe and count the type and number of eggs within clutches. We visually determined the presence or absence of the artificial and natural eggs. We recorded the number of eggs present in the nest throughout the experiment to determine if females were laying or incubating at the time of the experiment (see "Statistical analyses" below).

Robins normally reject eggs in less than 5 days following parasitism (Aidala et al. 2015); therefore, we recorded a response as a rejection if the artificial egg was absent from the nest within 5 days while the rest of the nest content remained being incubated, or as an acceptance if the experimental egg remained in the active nest for at least 5 days (Aidala et al. 2015). Nests showing signs of full or partial predation, including cracked eggshells, and absent natural eggs were excluded from the analyses. Ten nests were excluded from analysis: 2 nests were abandoned, 4 were predated and 4 clutches fully hatched in less than 4 days so that we could not confirm experimental egg acceptance. However, we note that this approach did not allow us to assess if recognition errors or rejection costs were elicited by our experiments in robins (Samaš et al. 2014). In addition to recording the presence of artificial eggs, we noted if robins were flushed at the moment when we introduced the experimental egg (Table 1) which might affect their responses (Hanley et al. 2015b).

### Experimental egg models

Experimental eggs were manufactured using a 2X 3D printer (MakerBot Replicator) using acrylonitrile butadiene styrene (ABS) following an established protocol to manufacture model cowbird eggs for experiments with robins (Igic et al. 2015). To ensure a smooth surface, each egg model was sanded and dipped in 100% acetone prior to painting. The artificial eggs

were designed to be within the size range of generic cowbird eggs ( $23 \times 18$  mm) as per the same model measurements used in Igic et al. (2015). This size and shape falls within the range of the natural cowbird egg's average of  $21.45 \times 16.42$  mm ( $n = 127$ ; largest  $25.40 \times 16.76$ , smallest  $18.03 \times 15.49$ ; Bent 1958). The experimental eggs were painted a uniform blue-green ground colour, closely mimicking the colour of natural robin eggs (Fig. 1a). To compare how similar our mimetic ground colour was to a real robin egg, we created a natural robin spectra measurement by averaging measurements of 10 real eggs found at our study site in abandoned nests (Fig. 1b), this value was later used for the "natural comparison" (Table 2). Chromatic contrast was calculated based on contrast between experimental models and experimental spots as well as contrast between experimental spots and natural robin egg spectra (Table 2). Experimental ground colour was different from a natural robin ground colour by an average chromatic contrast of 2.2 JND and an achromatic contrast of 0.15 JND (Fig. 1).

Each egg had 25 generic spots ranging from 0.2 to 14 mm<sup>2</sup> in size. The largest of the spots was located near the blunt pole of the experimental eggs so that it would be accurately measured by a spectrometer, the spots were gradually made smaller as they radiated from the large blunt pole spot. We used unique combinations of high-quality acrylic paints (Koh-i-Noor Hardtmuth A.s., České Budějovice, Czech Republic) to generate a mimetic ground coloration. Specifically, we mixed brown light (0640), khaki (0530) and turquoise (0460), paint brand was consistent with that used by Hanley et al. (2017). Then, when dry, eggshell spots were painted by hand on all experimental eggs with matching spot pattern, but with different spot colours (Fig. 1c). Each egg's spot colour was consistent within that same experimental eggs and was not repeated on any other egg, making each egg unique within the study (following Hanley et al. 2017). Colours were selected in attempt to express the full range of avian eggshell colour diversity (Hanley et al. 2015a; Hanley et al. 2017). We painted egg spots using mixtures of the same acrylic paints used to generate the ground coloration and in addition, we added additional acrylic paints (Grumbacher, Leeds, MA, USA) to darken the spots and emulate the greater pigment concentration found in spots. Specifically, we used burnt umber (C024) and raw umber (C172) to darken the spots. These paint mixtures generated colour and brightness contrast between each experimental egg's ground coloration and its spot colour (Fig. 1c).

### Colour analyses and modelling

We measured artificial egg reflectance spectra using an Ocean Optics USB 2000 spectrometer, a deuterium tungsten lamp (DT-mini) and a white reflectance standard (WS-1). We selected 10 model eggs at random and measured their

**Table 1** Summary statistics showing range of spot JND

	Natural robin ground colour JND		Experimental ground colour JND		Parasitized nests			
	Chromatic	Achromatic	Chromatic	Achromatic	Latency <sup>a</sup>	Flushed <sup>b</sup>	Initial clutch size	Final clutch size
Mean	2.94	2.13	3.65	2.13	1.50	0.73	3.14	2.57
SD	1.52	1.89	2.08	1.90	1.05	0.45	0.82	1.35
Min	1.18	0.23	0.18	0.18	0.00	0.00	2.00	0.00
Max	6.59	9.00	7.86	9.16	3.00	1.00	5.00	4.00

The natural robin ground JND is a calculation between the average robin ground coloration versus the experimental spot colour. The experimental ground colouration JND is a calculation between the experimental ground colour to the experimental spot colour. Also reported are statistics relating to rejection latency, flushing and clutch size of parasitized nests

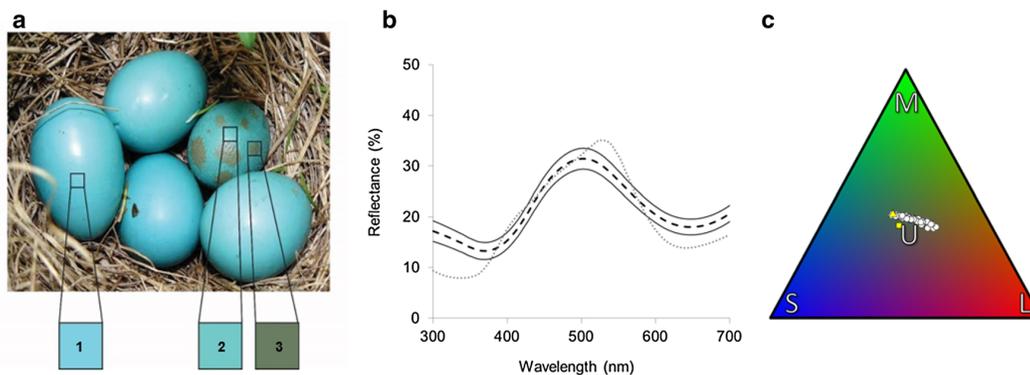
<sup>a</sup> Days until outcome

<sup>b</sup> With reference to the moment of parasitism

spectral reflectance to assess the blue-green ground colour that was applied to all eggs. Then, we measured the coloration of three large spots (with a surface area of approximately 14 mm<sup>2</sup>) located near the blunt pole of each egg. Raw reflectance spectra were smoothed using a locally weighted polynomial with a 0.25 nm smoothing span using the ‘pavo’ R package (Maia et al. 2013). These spectra were averaged, which resulted in a representation of the ground coloration and individual eggshell spot colours.

We used a neural noise-limited visual model (Vorobyev et al. 1998) to estimate host colour perception. These models were based on relative visual sensitivity data for the common (European) blackbird *Turdus merula* (Vorobyev et al. 1998; Govardovskii et al. 2000; Hart and Vorobyev 2005) because these parameters are unknown for the closely related, congeneric robin. Specifically, we modelled quantum catch using the four photoreceptor cones had peak sensitivities at

373.0, 453.5, 504.3 and 557.2 nm (Hart et al. 2000), with oil droplet cutoffs at 330, 414, 515 and 570 nm (Hart and Vorobyev 2005) and an experimentally derived signal-to-noise ratio such that the Weber fraction of the long-wave-sensitive cone was 0.1 (Olsson et al. 2015), while accounting for the relative abundance of cones and the principal member of the double cone (Hart and Vorobyev 2005) and assuming a standardized daylight illumination under bright conditions. Data on quantum catch were used to calculate both chromatic (hereafter chromatic contrast) and achromatic JND (hereafter achromatic contrast) (Vorobyev et al. 1998; Siddiqi et al. 2004) between the blue-green ground colour applied to all experimental eggs and the specific spot colour applied uniquely to each egg model. When these perceived differences between the ground colour and spot colour are <1, the differences would always be imperceptible, when the calculated difference is equal to 1, the difference would be just noticeable under ideal



**Fig. 1** **a** Experimental egg (top right) among a natural clutch of American robin eggs. Box 1 is a natural robin egg sample. Box 2 is the ground colour of the experimental egg model. Box 3 is a sample of spot colour, which varied on each experimental egg from more blue-green to more brown. **b** Spectra depicting the mean natural American robin colour (dashed) with the 95% confidence limits (solid), the grey dashed line is

the plot of the model egg's blue-green ground colour. **c** The tetrahedral colour space where the yellow triangle is the average ground colour of the model egg, the yellow square is the average ground colour of natural American robin eggs and the white circles are the range of experimental spot colours

**Table 2** Logistic regression model outputs predicting the rejection to artificial parasitic eggs colour

Predictor	Full model					Reduced model				
	Estimate	SE	LR $\chi^2$	<i>p</i>	VIF	Estimate	SE	LR $\chi^2$	<i>p</i>	VIF
Experimental ground colour										
Whole model	$(R^2 = 0.21, \chi^2 = 5.41, n = 44, p = 0.49)$					$(R^2 = 0.16, \chi^2 = 4.02, p = 0.04)$				
Intercept	-3.53	3.10	–	0.25	–	<i>-3.41</i>	<i>1.27</i>	–	<i>&lt;0.01</i>	–
JND brightness	0.07	0.23	0.09	0.77	1.24					
JND colour	<i>0.36</i>	<i>0.26</i>	<i>3.99</i>	<i>0.05</i>	<i>1.18</i>	<i>0.40</i>	<i>0.24</i>	<i>4.02</i>	<i>0.04</i>	–
Flushed <sup>a</sup>	0.61	1.42	0.84	0.36	1.35					
Breeding stage <sup>b</sup>	-0.31	1.42	0.22	0.64	1.83 <sup>d</sup>					
Initial clutch size <sup>c</sup>	0.02	0.75	<0.001	0.99	1.69					
Final clutch size	<0.01	0.45	0.03	0.86	1.59					
Natural ground colour										
Whole model	$(R^2 = 0.30, \chi^2 = 7.90, n = 44, p = 0.25)$					$(R^2 = 0.24, \chi^2 = 6.34, p = 0.01)$				
Intercept	-4.45	3.61	–	0.22	–	<i>-3.94</i>	<i>1.32</i>	–	<i>&lt;0.01</i>	–
JND brightness	0.09	0.23	0.13	0.71	1.20					
JND colour	<i>0.57</i>	<i>0.35</i>	<i>6.70</i>	<i>&lt;0.01</i>	<i>1.21</i>	<i>0.64</i>	<i>0.30</i>	<i>6.34</i>	<i>0.01</i>	–
Flushed <sup>a</sup>	0.65	1.55	0.86	0.35	1.46					
Breeding stage <sup>b</sup>	-0.24	1.77	0.21	0.64	1.90 <sup>d</sup>					
Initial clutch size <sup>c</sup>	0.11	0.82	0.02	0.89	1.69					
Final clutch size	0.03	0.47	0.08	0.77	1.62					

Significant predictors are in italic

<sup>a</sup> With reference to the moment of parasitism

<sup>b</sup> With reference to laying

<sup>c</sup> Natural clutch size at moment of parasitism

<sup>d</sup> Highest VIF being 1.90 for natural ground colour comparison and 1.83 for experimental ground colour

viewing conditions and when the calculated difference is >1, those differences would become increasingly noticeable as the value of the JND increases.

**Statistical analyses**

We used a binomial generalized linear model (GLM) framework to predict egg rejection based on two main predictors of interest: chromatic and achromatic contrast. This model controlled for several potential confounds including whether the host was flushed at the start of experiment (following Hanley et al. 2015b), nest stage (categorical: laying or incubation) and initial/final clutch size (continuous). Since cowbird hosts’ rejection data often include either many rejections or acceptances (Rothstein 1982), which may bias statistical analyses (Samaš et al. 2011), it is important to statistically correct the parameter estimates and calculated significances for rare events (Veazey et al. 2016). Here, we adopt one such method that is specifically designed to correct parameter estimates and significance values from logistic regression models (King and Zeng 2001); these models are otherwise identical to the standard binomial generalized linear models (GLM) that normally are used to model host response. We then ran a stepwise regression using backward elimination,

sequentially removing non-significant predictors from highest to lowest *p* values from the full model until the best-fitting model (hereafter reduced model) containing only significant predictors was reached. We present both the full (as recommended by Grafen and Hails 2002) and reduced models (as recommended by Forstmeier and Schielzeth 2010). We report Nagelkerke’s *R*<sup>2</sup> (Nagelkerke 1991) and the variance inflation factor (VIF) for all parameters. Moreover, the VIF for all parameters at the first and last step was less than two, which suggests a weak correlation among predictor variables at any point during the stepwise elimination process (see VIF Table 2). We ran this model with chromatic and achromatic contrast of the experimental eggshell background to spot colours and reran this model using chromatic and achromatic contrast of the natural robin eggs to the experimental spot colour. All statistical analyses were performed using R version 3.1.2.

**Results**

The full model was not significant for either natural or experimental comparison (Table 2); however, a backward elimination selection procedure found that, for both

comparisons (natural and experimental), a reduced model including only chromatic contrast significantly predicted egg rejection (Table 2). Robins were more likely to reject parasitic eggs when those eggs had spots with greater chromatic contrast, and this was true when using either experimental or natural ground colour spectra to calculate chromatic contrasts.

All rejected experimental eggs were ejected from the host nest within 3 days of parasitism (mean  $\pm$  SE =  $1.5 \pm 0.4$  days,  $N = 6$ ; Table 1). When chromatic contrast of these eggs was evaluated using a natural robin comparison, rejected eggs had an average of 4 JND (mean  $\pm$  SE =  $4.45 \pm 0.68$  JND,  $N = 6$ ). A spot with the highest degree of chromatic contrast (6.59 JND) resulted in a predicted rejection rate of 57% (Fig. 2b). Similarly, when chromatic contrast was evaluated using the actual experimental ground colour, we found that eggs with spots that contrasted highly with the experimental surface (7.86 JND) resulted in a rejection rate of approximately 43%, and rejected eggs had an average of 5 JND (mean  $\pm$  SE =  $5.21 \pm 1.12$  JND,  $N = 6$ ) (Fig. 2c).

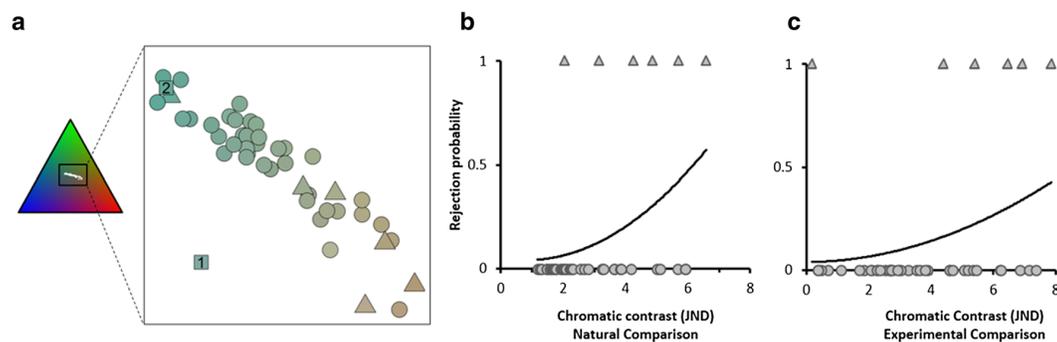
## Discussion

Our experiment was designed to test American robin response to experimental eggs with spots that changed in contrast along a gradient from blue to brown while the ground colour remained constant. We found that robins were more likely to reject artificial eggs when egg's spots had a greater degree of chromatic contrast to ground colour. This was true both when calculating chromatic contrast using a natural colour of robin eggshell and when calculating chromatic contrast using the ground colour of artificial experimental eggs.

While our experiment tested host rejection responses against internal changes within an experimental egg's

spot vs. background successfully, a statistical model comparing the experimental spot colour to an average natural robin egg colour also showed noteworthy results (Table 2). In the second comparison that used experimental spot colour against a natural robin colour (natural robin comparison), the chromatic colour of the spot significantly predicted rejection (Table 2). One explanation is that robins are not only discriminating based on a specific colour threshold within each potential foreign egg, but are also judging the egg based on the overall appearance of the egg in comparison to their own eggs (Moskát et al. 2010, Rothstein 1974). Possibly, the two stimuli together (the varied spot colour and varied ground colour) create an overall more strongly differing appearance relative to that of their own eggs and elicit a stronger rejection response than any of the stimuli in isolation (Spottiswoode and Stevens 2010; De la Colina et al. 2012).

Since our experimental ground colour differed from a natural robin egg by 2.2 JND (Fig. 1), our findings might have had different rates of rejections if we could more accurately match the experimental ground colours to natural host eggshell colours in each experimental egg. Figure 2 shows that natural robin egg's spectra are located farther away in colour space to each respective spot colour treatment than our experimental ground colour. In turn, the JND calculated using generic natural robin egg ground colours better predicted host rejection response (Table 2). Despite this, neither type of comparison achieved consistent rejection at 100% (Fig. 2). However, using artificial variation in model egg ground colours, Croston and Hauber (2014b) predicted a rejection rate of 45% at a chromatic contrast of 8 JNDs (figure 5 in Croston and Hauber 2014b). This theoretical prediction corresponds well with our empirical results of maximal predicted rejection rates



**Fig. 2** Circles represent accepted eggs, triangles represent rejected eggs, and squares represent natural robin ground colour and experimental ground colour. **a** The distribution of experimental spot colours plotted on tetrahedral colour space. Colours depicted are approximates for actual spot colour as converted from spectra to RGB space (Williams et al. 2007). Square 1 represents natural robin egg ground colour, while

square 2 represents the experimental ground colour. Spot RGB values were standardized by brightness. **b** Predicted probability illustrating increased probability of egg rejection as spot contrast increases from 0 to 8 JND using a natural comparison to calculate JND. **c** Predicted probability using experimental comparison to calculate JND

at 43% and 57% (Fig. 2); therefore, egg rejection decisions appear to have similar perceptual thresholds both considering discriminating colours within foreign eggs (i.e., discriminating between ground and spot colour of parasitic eggs) and between individual eggs (i.e., comparing ground coloration of own vs. experimental eggs). Our study could have better explored the experimental comparison had our experimental ground colour been a better match in chromatic JND to that of a natural robin egg, or if we had measured the actual coloration of every robin egg in the experimental nests in the field at the time of experimentation. Moreover, a systematic rather than random sampling procedure would have prevented the negative correlation between date and outcome (Hurlbert 1984); therefore, future work should consider sequential sampling of disparate or simultaneous testing of paired colours such that there would be no such correlation.

Consistent with past research on avian egg rejection cues and mechanisms (Stoddard and Stevens 2010), we found that chromatic contrast, but not achromatic contrast, significantly explained rejection responses. This applied to models comparing experimental spots to the experimental eggshell background colour or a natural robin colour. Therefore, it is likely that achromatic contrast is not a reliable egg recognition cue alone, but may act as a cue in conjunction with other egg traits. On the other hand, robins are known to use eggshell ground colour as an egg recognition cue (Rothstein 1982; Croston and Hauber 2014a, b; Dinets et al. 2015), and our study demonstrates that these hosts can also use the chromatic contrast between eggshell ground colour and spots for recognition decisions. This is in line with Rothstein's (1982) findings that contrasting spots can trigger robin egg rejection when placed on cowbird-sized model eggs with ground colour similar to the robin's own. Although chromatic contrast predicted host rejection responses, many of the rejected eggs had spots with a brown hue (Fig. 2). In nature, there are no known eggs that have spot patterns produced solely by biliverdin deposition, the compound responsible for creating a blue-green hue in eggshells (Kennedy and Vevers 1976). Recent research has found that hosts will reliably reject highly contrasted eggs, but only if they were contained within the natural eggshell colour gamut (Hanley et al. 2017). Our findings suggest that rejecter species may not recognize blue spots as spots, and those therefore elicit little to no response, implying that egg recognition in relation to spot pattern, like ground colour recognition, is tuned to the natural variation of avian eggshell spot colours (Hanley et al. 2017). Future work should examine the role of spot coloration in other host-parasite systems, including those where spot coloration is naturally variable within and between the individual hosts and parasites.

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**Compliance with ethical standards** All data for this study were acquired following approval by the Hunter College Institutional Animal Care and Use Committee (MH 2/16-T3).

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