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Research

Variation in multicomponent recognition cues alters egg rejection decisions: a test of the optimal acceptance threshold hypothesis

Daniel Hanley[1], Analía V. López[2], Vanina D. Fiorini[2,3], Juan C. Reboreda[2,3], Tomáš Grim[4,†] and Mark E. Hauber[5]

[1]Department of Biology, Long Island University – Post, Brookville, NY 11548, USA
[2]Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina
[3]Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA-CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina
[4]Department of Zoology, Laboratory of Ornithology, Palacký University, Olomouc 77146, Czech Republic
[5]Department of Animal Biology, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

DH, 0000-0003-0523-4335; AVL, 0000-0001-6791-8906; VDF, 0000-0003-0447-461X; JCR, 0000-0001-5136-4574; TG, 0000-0002-5775-6269; MEH, 0000-0003-2014-4928

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Author for correspondence:
Daniel Hanley
e-mail: daniel.hanley@liu.edu

The optimal acceptance threshold hypothesis provides a general predictive framework for testing behavioural responses to discrimination challenges. Decision-makers should respond to a stimulus when the perceived difference between that stimulus and a comparison template surpasses an acceptance threshold. We tested how individual components of a relevant recognition cue (experimental eggs) contributed to behavioural responses of chalk-browed mockingbirds, *Mimus saturninus*, a frequent host of the parasitic shiny cowbird, *Molothrus bonariensis*. To do this, we recorded responses to eggs that varied with respect to two components: colour, ranging from bluer to browner than the hosts’ own eggs, and spotting, either spotted like their own or unspotted. Although tests of this hypothesis typically assume that decisions are based on perceived colour dissimilarity between own and foreign eggs, we found that decisions were biased toward rejecting browner eggs. However, as predicted, hosts tolerated spotted eggs more than unspotted eggs, irrespective of colour. These results uncover how a single component of a multicomponent cue can shift a host’s discrimination threshold and illustrate how the optimal acceptance threshold hypothesis can be used as a framework to quantify the direction and amount of the shift (in avian perceptual units) of the response curve across relevant phenotypic ranges.

This article is part of the theme issue ‘The coevolutionary biology of brood parasitism: from mechanism to pattern’.

1. Introduction

Decision-making is critical for survival and reproduction [1], but animals often face challenging decisions involving discriminating between novel stimuli in a variety of contexts. Thus, the ability to produce flexible responses to novel situations is necessary for adaptive decisions [2]. One approach for making decisions between stimuli is to set a (flexible) threshold based on the similarity of the traits of each stimulus, such that differences greater than a threshold elicit a response. This ‘optimal acceptance threshold hypothesis’ was proposed almost 30 years ago [2] and has been used to predict decisions across a range of contexts: pollination [3], same-sex partnership [4], social insect nest-mate recognition [5] and foreign egg rejection [6]. In each case, stimuli vary and

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[†]Present address: Na Orátě 497, 783 61 Hlubočky, Czech Republic.

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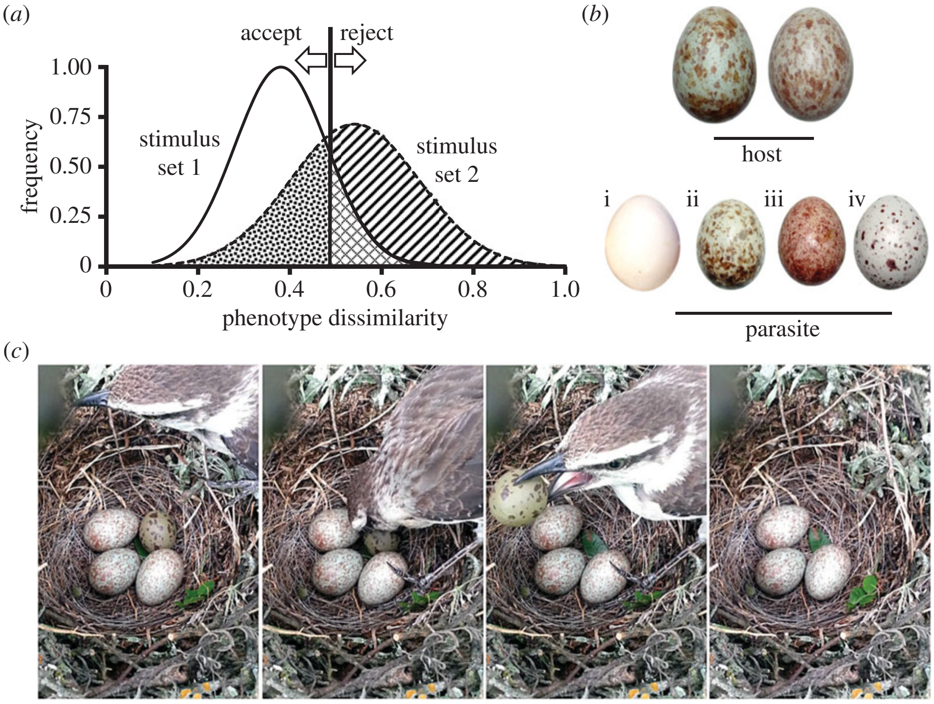

Figure 1. The optimal acceptance threshold hypothesis is a theoretical model used to understand the outcomes of discrimination decisions between (*a*) stimuli that vary relative to a template, e.g. in making decision between potential non-self and self (modified from [2]). Hosts of avian brood parasites can compare their own eggs (or an internal template of their eggs) with other eggs in their nests. When the difference between the template and the focal stimulus is greater than the acceptance threshold (solid vertical line), the theory predicts rejection by the host, but hosts should accept the stimulus when the difference is less than the threshold. Here we illustrate two sets of stimuli, such that stimulus set 1 represent eggs that should not be rejected (e.g. last laid eggs that appear different) and stimulus set 2 represents eggs that should be rejected (e.g. a parasite's egg). Thus, dotted areas represent acceptance errors, the crosshatched area represents rejection errors, and the linear hatched area represents correct rejection decisions. The chalk-browed mockingbird has a (*b*) blue–green spotted egg and is parasitized by the shiny cowbird, which has polymorphic eggs that vary in coloration and spotting (eggs i–iv). Mockingbirds tend to reject pure white, unspotted, cowbird eggs (i), while they accept other cowbird colour morphs: blue–green (ii) and both heavily and lightly spotted white eggs (iii and iv, respectively). However, the presence of spots may be sufficient to 'shift' an egg from the 'reject' to the 'accept' side of the acceptance threshold, because an additional matching feature would increase the uncertainty of the decision. This host rejects eggs through (*c*) grasp rejection, which we illustrate through a series of images showing a host returning to its nest, examining the egg, grabbing the egg and removing it (left to right). Photo credit: A. V. López.

the optimal choice is context dependent, i.e. an acceptable choice in one situation would not necessarily be an adaptive decision in another (figure 1*a*).

The optimal acceptance threshold hypothesis has found substantial traction within studies of avian brood parasitism. In such systems, brood parasites lay their eggs in hosts' nests, imposing the cost of parental care upon foster parents [7–9]. A host's ability to discriminate between its own and the parasite's egg, and appropriately respond to it, is an important evolved host defence against brood parasitism [7,8]. Hosts may respond to the presence of a brood parasite's egg in their nest by deserting the clutch, by burying the parasitic egg, or by ejecting it from the nest [10,11]. When making these decisions, hosts must balance the risk of accepting a parasitic egg (acceptance error) or mistakenly rejecting one of their own (rejection error).

Avian eggshell coloration and patterning can provide salient information for hosts to make rejection decisions [8]. Owing to the limited pigmentary basis for colour production in avian eggshells [12,13], the coloration of birds' eggs is constrained to fall along a gradient ranging from blue–green to brown within the avian perceptual colour space [14,15]. Most applications of the optimal acceptance threshold hypothesis to brood parasitism have assumed hosts base their decisions on absolute perceivable differences between their own eggs (or a template of them) and the foreign egg(s) in the nest [16]. This implies that hosts set thresholds along phenotypic extrema on each side of a phenotypic range ('multiple thresholds'), in this case, eggs that are very blue–green and brown. However, a recent study [17] on two species of thrushes (*Turdus* spp.) found that this was not the case. Instead, these hosts rejected brown eggs but accepted blue–green eggs, which would both be perceived as equally different from their own eggshell's colour. That is, they rejected eggs only on one side of their phenotypic range ('single threshold') [17]. Such a result could arise if hosts categorize eggshell colours during egg discrimination decisions, as has been demonstrated in birds in other contexts [18]. Regardless of the underlying mechanism, the optimal acceptance threshold hypothesis can be extended to accommodate a single threshold [19] and to identify novel decision rules that better explain host decisions. The natural avian eggshell colour gradient, varying from blue–green to brown [14], reduces trait dimensionality and, thus, makes bird egg coloration a valuable tool to explore such extensions of the optimal acceptance threshold hypothesis.

Colour is not the only important feature in egg discrimination decisions. The patterns generated through eggshell maculation, including spots and lines (hereafter, spots) are also important factors as they can generate unique egg signatures [20] that provide hosts with valuable information about egg ownership (own versus foreign) [21]. Hosts with spotted eggs are generally more likely to accept spotted foreign eggs, and hosts with unspotted eggs are generally more likely to

Figure 2. We painted pairs of three-dimensional printed [34] eggs along a phenotypic gradient of blue–green to brown, corresponding with natural variation in avian eggshell coloration across taxa [14], following [17]. After measuring the reflectance spectra of each egg, we (*a*) painted spots on one set and (*b*) left the other set unspotted. Here we depict 16 of 70 unique model eggs to demonstrate the range of colours and spotting patterns used. These eggs were placed within chalk-browed mockingbirds' nests (*c*–*h*) and the response of the host (either acceptance or rejection) was recorded over a 5-day period. Photo credit: A. V. López.

accept unspotted eggs [21,22]; in addition, spot colour, size, distribution and boundaries all provide valuable information to hosts [23,24]. The optimal acceptance threshold hypothesis predicts that when multiple components of the recognition cue are similar between stimuli (e.g. sharing colour and spotting rather than sharing either colour or spotting), their dissimilarity is decreased, and the likelihood of acceptance is greater [2]. For example, for a host with a spotted egg, the presence of spots on a foreign egg may be sufficient to shift it from the rejection side of the acceptance threshold to the acceptance side of the acceptance threshold, irrespective of that eggshell's coloration (figure 1). Thus, the optimal acceptance threshold hypothesis provides a framework for determining the relative contribution of individual components of multicomponent recognition cues to behavioural decisions.

We studied the chalk-browed mockingbird, *Mimus saturninus* (hereafter, mockingbird), which is a frequent host of the brood parasitic shiny cowbird *Molothrus bonariensis* (hereafter, cowbird) in South America. The mockingbird has a blue–green speckled egg (figure 1*b*), whereas the eggs of the cowbird are polymorphic [25], showing variation both in ground colour and in the density and distribution of the spots (figure 1*b*); cowbird eggs may be blue–green or white, or even brown if they are heavily spotted (figure 1*b*). Cowbirds reduce the fitness of the mockingbirds through puncturing host eggs [26–29] and through frequently engaging in multiple parasitism [30] (i.e. when more than one cowbird egg is found in the same nest). Mockingbirds actively defend their nests when parasites approach them [31] by vigorously mobbing the cowbirds [28], which can reduce the risk that the cowbird will puncture their eggs. In addition, mockingbirds also sometimes grasp-reject [28,32] (figure 1*c*) one specific cowbird egg morph: unspotted white cowbird eggs [22,32,33]. Since mockingbirds do not respond to variation within the colour range of spotted cowbird eggs [32], differential rejection responses to blue–green and brown eggs would be surprising. However, when choosing among a broader array of colours (e.g. the full extent of avian eggshell colours), it is feasible that mockingbirds will also preferentially reject browner model eggs. Such a finding would provide evidence that hosts in other genera also set a single acceptance threshold on one side of their eggshells' phenotypic range, as previously found in *Turdus* [17], and that this decision rule may be widespread among hosts of avian brood parasites.

To experimentally test colour-based egg discrimination in mockingbirds, we generated two sets of experimental egg models that varied identically along the span of natural eggshell coloration found across avian lineages (figure 2): from blue–green to brown [14]. Then, we added spots to one set of these experimental egg models. This generated a range of eggshell colours spanning the variation of real birds' eggshell colours, such that one set of eggs was more similar to the mockingbird eggs' own phenotype (spotted eggs) than the other set of eggs (unspotted eggs). By using this design, we tested whether mockingbirds applied a single threshold or multiple thresholds to colour-based egg discrimination decisions. If hosts applied multiple thresholds (set at both the bluer and browner side of their eggshell colour phenotypic range), we expected that mockingbirds would reject foreign eggs with a greater absolute perceived difference in eggshell colour from their own eggs. In this system, a host may face a parasite egg that is either bluer or browner than its own eggs (figure 1*b*); thus the application of multiple thresholds would prepare this host for either threat. Alternatively, if mockingbirds adhered to a single threshold decision rule, we expected that mockingbirds would reject brown eggs more than equally dissimilar blue–green eggs, as previously shown in other hosts [17]. This decision rule would expose hosts to threats from parasite eggs that are more blue–green than their own eggs, but may aid making rapid decisions when information is limited or uncertain [35,36] (e.g. when their clutch contains many parasite eggs). Finally, under either scenario, we expected that spotted eggs would be shifted towards the acceptance side (or region) of the acceptance threshold(s) relative to their unspotted counterparts, because for any given eggshell colour, spotted foreign eggs are more similar to the hosts' own eggs than unspotted eggs. By recording mockingbird egg rejection responses to these two sets of stimuli that vary along a natural phenotypic gradient, we quantified, in avian perceptual units, the contributions of both coloration and spotting toward egg rejection decisions.

2. Material and methods

(a) Experimental eggs

We ordered three-dimensional printed shiny cowbird egg models from shapeways.com (model: 6HVA2QYWW), using the previously described methods [34]. These models approximated the dimensions and mass of natural shiny cowbird eggs found at this site (*model egg* mean ± s.e.: (length × width) $22.6 \pm 0.03 \times 18.5 \pm 0.02$ mm, (mass) 3.3 ± 0.01 g, $n = 70$; *cowbird*: $23.2 \pm 0.1 \times 18.7 \pm 0.1$ mm, 4.26 ± 0.06 g, $n = 45$), which are both smaller than natural mockingbird eggs ($28.2 \pm 0.3 \times 20.3 \pm 0.2$ mm; 6.1 ± 0.1 g, $n = 20$). We hand painted pairs of three-dimensional printed eggs to generate eggs that varied in the ground coloration along the natural gradient of avian eggshells (figure 2), following a previously published method [17], such that each colour was used twice. On one set of eggs, we applied a spotting pattern using a mixture of high-quality acrylic paint (Grumbacher burnt umber and Mars black). Our goal was to produce a consistent spotting pattern on the egg, rather than to perfectly replicate the variation in cowbird spot patterns or colours (electronic supplementary material, figures S1 and S2). To that end, we randomly chose a shiny cowbird egg from this population to serve as a template for spotting patterns and transferred that on clear plastic. Then, we projected that spot pattern on each egg from the spotted set and used an identical mixture of acrylic paint to apply (by hand) a standardized spot pattern within and between eggs. The spots on our experimental egg models were darker (electronic supplementary material, figures S1 and S2 and table S1), larger (electronic supplementary material, figures S1 and table S2), and more evenly distributed across the egg's surface than the spots found on either the cowbird or host eggs (electronic supplementary material, figure S1 and table S2); however, spotting patterns on experimental eggs were consistent (for more details see electronic supplementary material, figure S1 and table S3).

(b) Field methods

Our research was conducted at Reserva El Destino, near the town of Magdalena, in Buenos Aires Province, Argentina (35°08′ S, 57°23′ W), from November 2014 to January 2015. Following previously established protocols for this species [37], a single randomly selected experimental egg was chosen and added to a host's nest, and those nests were monitored for 5 days after the introduction of each model egg ($n = 85$). Model eggs that were still present at the end of the 5-day monitoring period were considered accepted and those that went missing during this period were considered rejected. We excluded deserted ($n = 9$, all after cowbird egg punctures) and predated nests ($n = 6$) from analyses [38], leaving a final sample size of 70 nests. In addition, we recorded a variety of potentially relevant covariates, including whether the parent was flushed [39] during the experimental introduction (yes: 6, no: 64), the experimental date as ordinal day (relative to the first egg in our dataset, such that 12 November 2014 = 1) [40], nest age at the time of the experiment (relative to clutch completion, such that an experiment 1 day before clutch completion = −1) [41], and the number of host eggs (mean ± s.e.: 2.1 ± 0.7) and natural cowbird eggs (1.1 ± 1.0) at the time of the experiment [41]. Although it is possible that a natural pure white cowbird egg was laid and then swiftly rejected without our knowledge (other cowbird eggs are always accepted, see above), we checked nests daily and any such bias would be equally probable for any nest. Additionally, we could not control for the possibility that some individuals in our population had more experience with natural cowbird eggs than other individuals; however, previous research suggests that the mockingbird does not reject spotted eggs [30,32], so we expect consistent responses across mated pairs regardless of their experience.

(c) Colour measurements and avian visual models

We measured the coloration of experimental egg models ($n = 70$), using an Ocean Optics Jaz spectrometer with a pulsed xenon light source (Jaz-PX) and a white reflectance standard (WS-1). The spectral reflectance of each egg was measured six times, prior to the addition of any spots, and we used the average reflectance spectrum to represent the colour of each egg. We also measured abandoned natural eggs of hosts ($n = 51$) and their parasites ($n = 60$) using an Ocean Optics 2000 reflectance spectrometer with a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, Florida, USA), relative to a barium sulfate white standard, following [42,43]. The probe was consistently held 5 mm from the eggshell surface, at a 90° coincident normal measurement angle, for all measurements. Although we avoided spots wherever possible on natural eggshells, it was sometimes impossible to avoid including some fine spotting in our measurements, particularly on mockingbird eggs [14,44].

We smoothed each reflectance spectrum using a locally weighted polynomial prior to analysis. Then we applied a noise-limited discrimination model [45,46] to calculate the perceived difference in coloration and luminance (hereafter chromatic and achromatic contrasts, respectively) [45,47] between the average mockingbird eggshell coloration and that of each experimental egg, where the noise was calculated as the sum of neural and receptor noise [46]. As mockingbirds are phylogenetically placed among oscines with known ultraviolet (UV) visual sensitivity [48], we used the visual system of the average UV sensitive bird [49], and the blue tit, *Cyanistes caeruleus*, double cone sensitivity [50]. Because mockingbirds nest within and under scrub or tree foliage [51], these visual models assumed a filtered forest light [52] scaled to simulate bright viewing conditions [53]. These analyses resulted in estimates of perceivable differences in just noticeable difference (JND) units, where values less than 1.0 would not be noticeably different, values of 1.0 would be just noticeable under ideal conditions, and as values become increasingly larger than 1.0 those differences become increasingly discernible. These JND thresholds are theoretical, such that actual discriminability thresholds may differ [54]; however, the exact threshold does not affect our findings, as our goal was to generate a range of discriminable differences.

We painted eggs such that the ground colour between spotted and unspotted eggs of each pair was similar (chromatic contrast = 0.26 ± 0.03 JND, achromatic contrast = 0.52 ± 0.05 JND, $N = 70$). By contrast, our experimental colours expanded the natural variation in eggshell colour [14,17] and ranged from similar to noticeably different from natural mockingbird (chromatic contrast range = 0.76 to 8.32 JND; achromatic contrast range = 0.01 to 13.78 JND) and cowbird eggshell colours (chromatic contrast range = 1.85 to 8.62 JND; achromatic contrast range = 3.51 to 16.81 JND). However, every real host egg was similar to at least one experimental egg model's colour and luminance (mean ± s.e. of the closest match for chromatic contrast = 1.44 ± 0.05 JND; achromatic contrast = 3.52 ± 0.26). These differences were similar to the average perceivable differences between host and cowbird eggshell colour (chromatic contrast = 1.34 ± 0.04 JND, $t_{100} = -1.49$, $p = 0.14$) and luminance (achromatic contrast = 3.63 ± 0.24, $t_{100} = 0.29$, $p = 0.77$), respectively.

We also calculated each model egg's position within the avian tetrahedral colour space [55,56] to determine if mockingbirds would perceive each experimental egg as more blue–green (i.e. closer to the short-wave sensitive photoreceptor) or browner (i.e. closer to the long-wave sensitive photoreceptor) than their average egg colour. Although we recognize that abandoned eggs may fade [57], we assumed that these abandoned eggs provided a reasonable estimate of host eggshell colour; mockingbirds do not respond to the vast majority of cowbird parasitism, and it is unlikely that those laying a particular colour egg would produce a particular type of response. To test egg rejection along the blue–green to brown eggshell colour gradient, the JNDs between bluer eggs and the average host egg were multiplied by −1, while JNDs between browner eggs and the average host egg were multiplied by 1. All colour and perceptual modelling analyses were conducted using the ‘pavo’ package [58] in the programing language R.

(d) Statistical analyses

We examined differences in rejection rates between spotted and unspotted eggshells first using Fisher's exact tests, which we report through their associated odds ratios (OR) and 95% confidence intervals (CI). We then examined mockingbird rejection behaviours using a generalized linear model (GLM), using the ‘*glm*’ function in the ‘*stats*’ package within the R environment for statistical computing [59]. Since host responses were either ‘accept’ or ‘reject’, we used a binomial distribution with a logit link function. We calculated the significance of whole models by comparing each parametrized model with a null model including just the intercept [60]. We generated two models to test whether host responses were better explained by the multiple threshold rule or the single threshold rule. First, to test the multiple threshold rule, we predicted host response by absolute perceivable differences in coloration (chromatic contrast, continuous in JND units), the presence or absence of spots (categorical: yes or no), and the number of cowbird eggs at the time of the experiment (continuous). Here, large chromatic contrast values correspond with eggs that were either bluer or browner than the average mockingbird egg. Second, to test the single threshold rule, we constructed an identical model but included directional colour differences varying from blue–green to brown (directional colour differences, continuous in JND units) instead of chromatic contrasts. Here, large directional colour differences correspond with eggs that are browner than the average mockingbird egg.

In addition, we performed an additional analysis (see supplementary material, table S4) to explore the potential role of a range of covariates by constructing a global model including the variables above as well as a range of covariates: whether the host was flushed from the nest (categorical: yes or no), nest age at the time of the experiment (continuous, hatching date = day 0), the number of host eggs (continuous) at the time of the experiment, and the experiment date (continuous). This model included an interaction to explicitly consider the possibility that hosts responded differently to directional colour differences (e.g. along a different response curve) between spotted and unspotted eggs. For example, hosts may accept all spotted eggs (regardless of their colour), but they may reject brown unspotted eggs and accept blue–green unspotted eggs. We then performed a backward-elimination selection procedure, by sequentially removing the covariate with the largest non-significant p-values, and rerunning the model with the remaining covariates until a final model including only significant predictors remained [61].

All continuous variables were centred and scaled prior to analyses and to aid the comparison of individual parameters and their interactions [62]. As an estimate of the goodness of fit, we report Nagelkerke's R^2 [63] and the corrected Akaike's information criterion ($\mathrm{AIC_c}$) [63,64] for each model. We compared the predictive power of multiple models using $\mathrm{AIC_c}$-based evidence ratios [65,66]. Unfortunately, owing to the colours of available paints, and the properties of subtractive colour mixing, despite our best efforts, avian perceived brightness covaried across the colour gradient such that browner eggs were darker; so achromatic contrast and directional colour differences were positively correlated ($r = 0.82$, $\mathrm{CI}_{0.95} = 0.73$ to 0.89, $n = 70$, $p < 0.0001$). Therefore, to avoid multicollinearity, we did not include achromatic contrasts in our statistical models. Instead, to determine whether host responses were better predicted by achromatic contrast or directional colour differences, we examined the independent effects of these predictors to explain host responses using a subsampling procedure. Specifically, we randomly sampled a subset of the rejection dataset ($n = 35$) 1000 times, and for each sample we conducted separate GLMs predicting host response by either achromatic contrast or directional colour differences. We then compared the sampled standardized effect estimates from these models using two-tailed paired t-tests. We also compared the sampled p-values using similar t-tests. As an additional method of comparison, and to parallel the analysis with achromatic contrasts, we provide similar tests to evaluate the relative importance of perceived differences between foreign and host eggs in terms of absolute perceived colour differences (i.e. chromatic contrast) and directional colour differences (i.e. how much bluer or browner a foreign egg is relative to that of the host). All analyses were conducted in R v. 3.1.2 [59]. All data are presented as mean ± standard error.

3. Results

At our study site, 67% of the monitored mockingbird nests were naturally parasitized by cowbird eggs; the subset of parasitized nests had a parasitism intensity of 1.6 ± 0.1 cowbird eggs per nest (range: 1–4) and we did not detect any rejection of natural cowbird eggs in this study. We detected a 45.7% rejection rate for experimental eggs ($n = 32$ of 70 eggs); however, unspotted eggs were rejected in 58.3% of trials ($n = 21$ of 36 eggs) and spotted eggs were rejected only 32.4% of the time ($n = 11$ of 34 eggs). These differences in egg ejection responses demonstrate that the likelihood of a mockingbird rejecting an unspotted egg is greater than the likelihood of rejecting a spotted egg (OR = 0.35, $\mathrm{CI}_{0.95} = 0.11–1.01$, $p = 0.03$), which corresponds to the results of separate GLMs (table 1).

Contrary to our expectations of host responses under a multiple threshold decision rule, we found that host

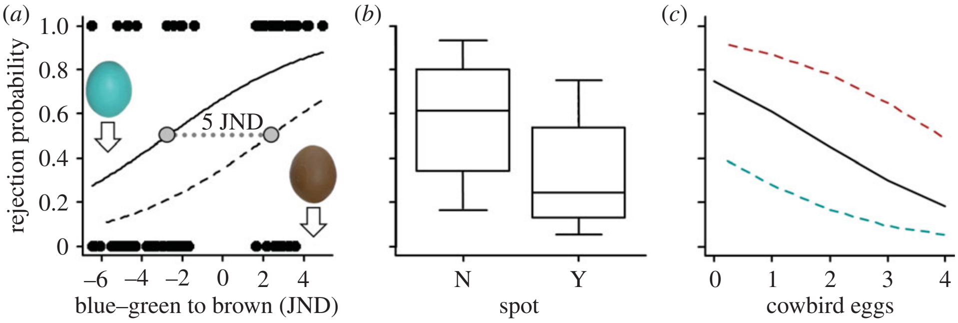

Figure 3. The probability of rejecting an experimental egg as predicted by the main effects: (*a*) variation in experimental egg colour, (*b*) spot absence (N) or presence (Y), and (*c*) the number of cowbird eggs in the nest at the time of the experiment. A binomial GLM predicted that mockingbird (*a*) behavioural responses (black dots; accept = 0.0, eject = 1.0) were biased toward rejecting brown unspotted (solid line) and spotted (dashed line) model eggs; however, the behavioural responses to these model eggs differed such that they were likely to reject an unspotted foreign egg at the 50% level if it was 2.71 JND bluer than their own, but if the egg was spotted it would need to be 2.31 JND browner (see grey circles connected by grey dotted line) than their own egg to illicit the same response. In general (*b*), unspotted model eggs were more likely to be rejected than spotted eggs. Similarly, mockingbirds were (*c*) more likely to respond to experimental parasitism when they had fewer natural parasitic cowbird eggs in the nest. Here we represent predicted rejection probability of model eggs versus the number of natural cowbird eggs for an egg model of the average colour (solid line), as well as for egg models with colours below (the lower dashed blue–green line) and above (the upper dashed brown line) the lower and upper 95% confidence limits for sampled colour variation. As the number of real cowbird eggs increased in the clutch, and dominated the nest, hosts were less likely to reject the experimental model egg. (Online version in colour.)

Table 1. Generalized linear models predicting the probability of mockingbirds rejecting foreign egg models based on either multiple or single thresholds. We present Nagelkerke's R^2 and AIC$_c$ for whole models, parameter estimates representing the change in log-odds and their associated standard errors (s.e.), the lower and upper limits of the 95% confidence interval (LCL and UCL), a measure of standardized effect (*z*-score) and variance inflation factors (VIF) for all parameters. 'Directional colour' represents the change in log-odds of rejecting egg models that are 1.0 just noticeable difference (JND) more blue–green (negative) or browner (positive) than the average mockingbird egg. 'Chromatic contrast' and 'achromatic contrast' represent the difference in perceivable coloration and brightness between egg models and the average mockingbird egg, respectively (see Methods for full details). 'Cowbird eggs' represents the number of cowbird eggs at the time of experimentation. 'Spot' is coded as unspotted (0) or spotted (1). Significant models and effects are italicized.

decision rule	predictor	estimate	s.e.	LCL	UCL	*z*	χ^2	d.f.	*p*-value	VIF
multiple	*full model* ($\chi^2 = 11.80$, $R^2 = 0.21$, AIC$_c = 93.34$, $p = 0.008$)									
threshold	intercept	*1.78*	*0.85*	*0.19*	*3.56*	*2.09*	—	*1*	*0.04*	—
	chromatic contrast	−0.22	0.20	−0.63	0.17	−1.09	1.21	1	0.27	1.02
	spot	*−1.20*	*0.53*	*−2.28*	*−0.18*	*−2.25*	*5.36*	*1*	*0.02*	*1.03*
	cowbird eggs	*−0.63*	*0.29*	*−1.23*	*−0.09*	*−2.16*	*5.21*	*1*	*0.02*	*1.02*
single	*reduced model* ($\chi^2 = 21.34$, $R^2 = 0.35$, AICc $= 83.80$, $p < 0.0001$)									
threshold	intercept	0.43	0.39	−0.33	1.22	1.09		1	0.28	—
	directional colour	*0.91*	*0.30*	*0.36*	*1.53*	*3.07*	*10.76*	*1*	*<0.01*	*1.04*
	spot	*−1.31*	*0.58*	*−2.51*	*−0.21*	*−2.25*	*5.46*	*1*	*0.02*	*1.05*
	cowbird eggs	*−0.64*	*0.29*	*−1.25*	*−0.09*	*−2.20*	*5.26*	*1*	*0.02*	*1.03*

responses were predicted by spot presence and the number of cowbird eggs at the time of the experiment, but not chromatic contrast (table 1). By contrast, a similarly constructed model supported our expectations of host response under a single threshold decision rule. Specifically, host responses were significantly predicted by directional colour differences (figure 3*a*), spot presence (figure 3*b*) and the number of cowbird eggs at the time of the experiment (figure 3*c*). In fact, evidence ratios suggest that this model predicted host behaviour 118 times better than one based on absolute perceptual distances, i.e. chromatic contrast (table 1). This model was corroborated through a stepwise selection procedure that found that host responses were best predicted by directional colour differences, whether the experimental egg was spotted and how many cowbird eggs were present in the nest at the time of the experiment (electronic supplementary material, table S4). Furthermore, our resampling procedure found that directional differences in colour (i.e. how much bluer or browner the experimental egg was relative to that of the host) explained host responses significantly better than chromatic contrast (directional colour $\beta = 0.46 \pm 0.002$, chromatic contrast $\beta = -0.18 \pm 0.008$, $t_{999} = 102.70$, $p < 0.0001$; directional colour $p < 0.0001 \pm {<}0.0001$, chromatic contrast $p = 0.54 \pm 0.008$, $t_{999} = -63.15$, $p < 0.0001$; see standardized effects in table 1). Importantly, we found no significant interaction, suggesting that the application of eggshell spots shifted eggs to the acceptance side of the acceptance threshold, but the mockingbird's response to colour variation otherwise followed a similar sigmoidal function (figure 3*a*). These findings demonstrate that mockingbirds preferentially rejected brown eggs (both spotted and unspotted), and were more permissive of spotted eggs and more restrictive of unspotted eggs. Specifically, the colour that would result in an experimental egg model being rejected 50% of the time differed by 5.0 JND between spotted and unspotted eggs (figure 3*a*). Although directional colour differences were related to perceived brightness, our resampling analyses demonstrate that directional colour differences significantly predicted host response but achromatic contrasts did not (directional colour $\beta = 0.46 \pm 0.002$, achromatic contrast $\beta = -0.24 \pm 0.01$, $t_{999} = 86.93$, $p < 0.0001$; directional colour $p < 0.0001 \pm {<}0.0001$, achromatic contrast $p = 0.50 \pm 0.009$, $t_{999} = -57.05$, $p < 0.0001$; electronic supplementary material, figure S3). In other words, although browner egg models were also darker, there is no statistical evidence that this affected the likelihood of egg rejection, as directional colour differences predicted host responses better than perceived differences in brightness.

4. Discussion

Optimal acceptance threshold theory has been successfully applied to predict the direction of shifts in egg rejection decisions in several different hosts of avian brood parasites [6,67,68]. However, no study has quantified the extent to which individual components of a recognition cue (such as colour or spotting) can shift a stimulus toward the acceptance or rejection side of the acceptance threshold. Here we measured how mockingbirds responded to spotted or unspotted model cowbird eggs coloured along a gradient corresponding with natural avian eggshell coloration (figure 3). We discovered that host responses were not explained by the absolute perceived differences between their own and foreign eggs, which is often assumed by tests of the optimal acceptance threshold hypothesis. Instead, our findings demonstrate that mockingbirds in South America, similar to egg rejecter *Turdus* thrushes in North America and Europe [17], are biased toward rejecting brown eggs over blue–green (figure 3). However, as predicted by the optimal acceptance threshold hypothesis, when the discrimination task was made more challenging by experimentally increasing the similarity between foreign eggs (through the addition of spotting), rejection responses became less likely. Specifically, eggshell spots made these foreign eggs more similar to the hosts' own phenotype (for every particular eggshell colour tested in our experiment). As predicted, the addition of spots shifted eggs to the 'acceptance' side of the acceptance threshold.

Tests of the optimal acceptance threshold theory generally assume that hosts will respond to absolute perceived dissimilarity between the hosts' own and foreign eggs [2]; therefore, it is surprising that hosts rejected eggs browner than their own and accepted eggs more blue–green than their own, even if these had the same absolute perceived differences from the bird's own egg colour (or its internal template representation [69]). Although it is possible that dark brown eggs are more cryptic and therefore more difficult to detect in the nest [70], we found that these eggs were rejected at higher rates, suggesting this alternative is unlikely in this case. The data from the current study are consistent with the findings of a previous study examining the responses of European blackbirds, *Turdus merula*, and American robins, *T. migratorius*, to egg models coloured along the same blue–green to brown eggshell colour gradient [17]. In addition to providing meta-replication in a third species, our new findings demonstrate meta-replication of these patterns in a third continent (South America, in addition to Europe and North America) and a third brood parasite species (the shiny cowbird, in addition to conspecific European blackbird and interspecific brown-headed cowbird *Molothrus ater* parasitism [17]). These findings consistently challenge the long-held assumption that hosts use absolute perceivable colour differences between their own and the foreign eggs (reviewed in [16]). Instead, such decisions could be produced if hosts use colour categorization; if hosts' internal templates are bluer than their own eggs are; or if hosts use a currently unknown cognitive mechanism for egg discrimination [71]. Thus, the underlying mechanism of colour-based egg discrimination, across diverse host species in different types of brood parasitism, deserves greater attention. We encourage the development of novel hypotheses as well as new and more consistent methodologies [72] for research examining the phenotypic and cognitive bases of egg rejection decisions.

The mockingbird's main defence against parasitism is mobbing, although they are generally unable to prevent parasitic egg laying or to reject parasitic cowbird eggs once laid [28]. The exception, however, is that mockingbirds will predictably reject natural, unspotted white cowbird eggs [22], which may be because they are unlike their own eggs in two components of eggshell appearance: coloration and spots [22]. Although this suggests that mockingbirds do not discriminate eggs based on colour, we found that mockingbirds have strong rejection responses to (even spotted) foreign egg models browner than their own. Thus, mockingbirds do have the ability to discriminate models of parasitic eggs based on their colour alone, even though when confronted with natural parasitism these discrimination abilities are generally not exercised. In addition, we demonstrate the importance of both eggshell colour and spotting on rejection decisions in this species. We found that a spotted egg could be up to 5.0 JND browner than an unspotted egg but still have the same likelihood of being rejected (figure 3*a*). In fact, for the brownest pair of eggs (which were the most likely to be rejected; figure 2), the unspotted brown egg resulted in a higher rejection rate (greater than 80%) than the spotted egg (approx. 60%; figure 3*a*). By contrast, the pair of eggs that were the most blue–green (figure 2) both had low rejection rates, but again, the unspotted blue–green eggs were more likely to be rejected (approx. 30%) than the spotted blue–green egg (less than 10%; figure 3*a*). Our findings suggest that this host has not yet adapted the ability to discriminate fine-grained differences in eggshell patterns, but instead uses eggshell features as an all-or-nothing cue. Although it is possible that artificial eggs with spots were more cryptic and thus harder to detect [73] in mockingbird nests, this is unlikely because rejection probability was predicted by eggshell colour variation within spotted eggs (the phenotype that is theoretically most cryptic, spotted brown eggs, was rejected at the highest rates). Moreover, the spotting patterns on mockingbird eggs differ from those of the cowbird (figure 1*b* and electronic supplementary material, figure S1*c*), which suggests that the amount of spotting and the exact spotting pattern do not necessarily impact host response. Nonetheless, we encourage future research to explore whether and how a host's perception of fine-grained spot patterns may lead to shifts in rejection decisions [20].

Within our study area, shiny cowbird females lack territoriality [74] and mockingbird nests are often parasitized by multiple cowbirds [28,75]. When hosts are parasitized multiple times, discriminating the parasitic egg from the host's own becomes more challenging and requires greater perceivable differences in eggshell phenotypes to elicit a response [76,77]. This was experimentally demonstrated in great reed warblers, *Acrocephalus arundinaceus*, [69] and the tawny-flanked prinia, *Prinia subflava*, [76], which are hosts to the common cuckoo, *Cuculus canorus*, and the cuckoo finch, *Anomalospiza imberbis*, respectively. Similarly, we found that when mockingbirds faced a greater number of natural shiny cowbird eggs in their clutch, they were less likely to reject the foreign egg models. Thus, our results suggest that engaging in multiple parasitism and laying spotted eggshells provide a substantial advantage to shiny cowbirds to avoid egg rejection by chalk-browed mockingbirds, and therefore multiple parasitism and eggshell spotting are likely to be under strong selective pressure in this shiny cowbird population. Similarly, cowbirds that lay spotted blue–green eggs in mockingbird nests should have a selective advantage over those laying other morphs; nonetheless, several other egg morphs persist in this population [30,78]. We encourage future research to examine whether colour-biased egg discrimination could play a role in regulating natural cowbird egg morphs.

We demonstrated that mockingbird responses are biased toward rejecting browner eggs, rather than being based upon absolute perceivable differences in eggshell colour. Contrary to the assumptions often applied to the acceptance threshold hypothesis, this host did not respond to the degree of dissimilarity between their eggs and foreign eggs *per se*. Instead, this host rejected brown eggs but accepted equally dissimilar blue–green eggs. These patterns suggest important and unexplored aspects of co-evolutionary dynamics within the chalk-browed mockingbird–shiny cowbird system, and host–parasite dynamics more generally. Moreover, these findings suggest that this unnecessary, though often applied, assumption to the acceptance threshold hypothesis is limiting our full exploration of its versatility. We illustrate that decision-making is not always adequately predicted simply by perceptual distances and that the acceptance threshold hypothesis should not be restricted by such assumptions. Additionally, as predicted by the acceptance threshold hypothesis, when the uncertainty of that decision was altered by the experimental addition of spots (i.e. reducing the overall trait dissimilarity, figure 1*a*) or the increased number of natural parasitic eggs (figure 3*c*), the likelihood of egg rejection was reduced. By using a simple experimental design with treatments (spotted or unspotted) varying along the same continuous range of ground colours, we were able to effectively measure (in avian perceivable units) the degree to which spots contributed to eggshell appearance.

Colour-biased responses might be expected if hosts use colour categorization for egg discrimination tasks, as has been demonstrated in birds in other contexts [79]. Previous research [17] found that hosts were more adept at differentiating differences between blue–green and brown eggs than within either colour category, despite the absolute perceivable differences within or between groups [17], which is a defining feature of categorical perception [80–82]. In this study, birds similarly produced differential responses to eggs more blue–green or browner than their own. Together, these findings suggest that hosts may use colour categorization as the basis for rejection decisions [17], which would potentially allow hosts, even naive hosts, to make rapid decisions [18] in the absence of other information or experience detecting parasitic eggs or under conditions when the information is uncertain [35,80,83]. Although further research is required to confirm the underlying mechanism explaining these behaviours, our research does demonstrate that decision boundaries can be set throughout the phenotypic range and that specific components of a multicomponent cue can shift the position of those decision boundaries. This finding, from a wild population, has important implications for future investigations to determine how decision boundaries are set when information is uncertain [36]. Lastly, given these findings, we recommend that the theoretical models we use to study animal decision-making should be as flexible and varied as the decision rules employed by the decision-makers themselves.

Ethics. The study was conducted with the permission of the Provincial Organism for Sustainable Development (OPDS, Buenos Aires, Argentina; permit no. 202/12-O.P.D.S.) and complies with the current laws of Argentina.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. D.H. and M.E.H. designed the field study. V.D.F. coordinated the study in Argentina and supervised the field research. A.V.L. collected field data. J.C.R. supervised the general project in Argentina. D.H. conducted the statistical analyses. T.G. provided advice throughout. DH wrote the initial draft. All co-authors contributed to writing and editing.

Competing interests. We declare we have no competing interests.

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Variation in multicomponent recognition cues alters egg rejection decisions: a test of the optimal acceptance threshold hypothesis

Daniel Hanley, Analía V. López, Vanina D. Fiorini, Juan C. Reboreda, Tomáš Grim, and Mark E. Hauber

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ELECTRONIC SUPPLEMENTARY MATERIAL

Contents:

Extended materials and methods

(*a*) General methods for quantifying spots

To examine if the spots we applied to experimental eggs were consistent, and to determine how they compared to spots on natural parasite and host eggs, we quantified mean spot size, spot distribution, and spot intensity [S1] from standardized photographs. In these photographs, host eggs, parasite eggs (if any), and spotted experimental eggs were photographed together with the eggs from the same nest on a standardized background (figure S1). In total, 279 eggs images were analysed.

All images were taken on a Panasonic DCM-FZ8 at 100 ISO using automatic settings using a flash. Only a subset of nests were accessible for such photographs (N = 67); therefore, these metrics serve to quantify our artificial spots rather than producing metrics to include in our main statistical analysis (see main text). To avoid pseudoreplication, we ran analysis of variance and flexible discriminant analysis using a subsampling procedure where we selected, when available, a single host, parasite, and experimental egg from each nest. Each analysis was repeated 100 times, and we present the mean±s.e. for those analyses. Our goal was to determine the significant pairwise differences between these three egg types, therefore we report the results of Tukey honest significance tests. We also ran a flexible discriminant analysis [S2], using all eggs as our "training set" and we report the percentages of correct versus incorrect classifications. To place these parameters on the same scale, all data were normalized, such that the minimum was set to zero and the maximum set to one, prior to plotting and analysis.

The original photographs were shot and saved in JPG format, and we recognize that this format is a 'lossy format' (a compression format) that will add some noise to our spot quantification analyses [S2]. However, we argue that in this limitation should not negatively impact or bias our results. Firstly, the noise introduced by this format occurs at the pixel level which equivalent to 0.002±<0.0001 mm^2 and unlikely noticed by our host because all available evidence suggests their visual acuity is, at most, half as acute as our own [S3,S4]. Secondly, our goal with these analyses is to quantify the gross differences between eggshell spots in host, parasite, and experimental eggs rather than assess perceivable differences between these eggs. Thirdly, if spots were inconsistently applied to experimental eggs, and if birds respond differently to those spot patterns, any relationship between the simple spotting parameter (yes or no) and host behaviour would be relatively conservative. Nonetheless, to minimize potential bias each set of eggs was photographed and subsequently analysed identically, enabling us to quantify the gross differences between the spots of hosts, parasites, and the experimental eggs presented in this study.

We also measured spots from our experimental egg models using a spectrophotometer and used avian visual models (see Methods from main text for details) to quantify chromatic and achromatic contrast between these spots and those found on both host and cowbird eggs (see below)

(*b*) Initial processing

Each photograph was white balanced and size calibrated using ImageJ, then each egg was isolated from the photograph and labelled with the nest ID, order in the photograph (1 to *n*), and whether it was a host, cowbird, or experimental egg model and stored as a separate image. Then, for each egg, we calculated the total area (mm^2) and used the 'subtract background' function (https://imagej.net/Rolling_Ball_Background_Subtraction) using a 2.5 mm pixel radius separately for all three channels (red, green, and blue) separately to reduce the influence of shadows caused by egg curvature; this technique essentially subtracts a local average from the original image, such that spots are darker relative eggshell ground coloration in brighter and more shaded parts of the egg. We then made these images binary, setting black values to 255 and white values to 0 (note images figure S1*b* are depicted with an inverted LUT), and saved this image in TIFF format for each egg. We then applied the 'analyze particles' tool to provide spot area and coordinates representing each detected their centre of mass. We quantified spot size, distribution, and intensity using the steps outlined below. Our goal was to provide a comparable approach for quantifying spot patterns, not necessarily to detect every spot (some light spots or small spots may escape detection on all eggs); however, we visually inspected the output and removed any eggs from the dataset where spot detection was entirely unsuccessful (i.e., spots were present but were not detected).

(*c*) Spot size

For each egg we quantified mean spot size (S) as a proportion of spot area (mm^2) to total egg area (mm^2) using the following equation:

$$S = \frac{\left(\sum_{i=1}^{n} A_i / A_e\right)}{n}$$

Where A_e represents total egg area, A_i represents the area of the i^{th} spot, and n represents the number of spots detected on the egg.

(*d*) Distribution

We quantified distribution as the product of coverage and mean intra-egg spot distances. We quantified spot coverage (c), using the same nomenclature as above, for each egg as follows:

$$c = \frac{\sum_{i=1}^{n} A_i}{A_e}$$

Calculated in this way an egg entirely occluded by spots would have a value of 1 because the sum area of its spots would equal the total area of the egg. We then calculated the median Euclidean distances between detected spots' centres of mass using the 'dist' function in R, which calculates pairwise distances between points. To do this, we negated the set of pairwise Euclidean distances between all spots found within an egg (d_n) for each egg, because larger Euclidean distances indicate sparser coverage. This made all values less than or equal to zero, therefore we added the absolute value of the minimum distance found in the set of all eggs to each value, such that the resulting set of distances were positive and comparable. Then to calculate spread (s), we normalized these (positive and comparable) distances relative to the largest value in the resultant set of distances so eggs with very small inter-spot distances had values close to one, while eggs with very large inter-spot distances had values close to zero. Finally, we calculated distribution (D) as the product of spread and coverage, such that an egg entirely occluded by close spots would have a value of one, while an egg with large blotchy spots near one pole would have a value closer to zero.

(*e*) Intensity

To calculate relative spot intensity, we used subtracted our original image from the binary image delineating eggshell spots. Because spots were classified by the largest values in the binary image (black = 255, see above) and by the smallest values in the original image (black = 0), the grey values in the resultant image had a bimodal distribution: all classified spots are negative, all areas designated as ground colour are positive. The resultant images were saved and we used the 'Save XY Coordinates' function, removing background pixels, to save the resultant values of each pixel. Spot intensity (I) was calculated as,

$$I = \left\{\frac{(i + 255)}{255} : i \leq 0\right\}$$

In this equation we adjusted the grey value back to its original scale (0 to 255, where black = 0) for only areas designated as spots. These values captured variation in eggshell spot intensity as found in both superficial and subcutaneous spotting. We report the median intensity.

(*f*) Quantifying avian perceived spot colour and luminance

In addition to measuring the ground colour of hosts and parasites (see Methods), when time allowed, we also measured the spectral reflectance of natural spots when they were large enough to measure with our spectrometer. Measuring spot coloration, or assessing response relative to spot coloration, was not our intent because hosts appear to respond strictly to the presence or absence of spots [S5–7]; however, we present these data to provide further details about our model eggs and their creation, which may inform future analyses. The mixture of paint used for spots was identical for all spots, and therefore we measured three spots from a single blue-green egg model that we painted larger than our normal spot pattern (described above). This ensured that the measurements of spot coloration did not include an admixture of spot and ground colour. We processed spot colour for all egg types through the same visual models (see Methods) and report analysis of variance testing the differences in chromatic and achromatic contrast between specific comparison types (e.g., experimental egg model to experimental egg model, experimental egg model to host egg, experimental egg model to cowbird egg, host egg to host egg, and host egg to cowbird egg).

Extended Results

The spots for five spotted experimental eggs were not successfully detected spots and therefore were excluded from our analyses. In one case, a blade of grass obstructed view of the egg and was detected as a spot, while the other four eggs were dark and the spots escaped detection. We also excluded five host eggs, from two nests (three eggs from one, and two from another) that were also too dark to successfully detect spots. In these cases, the natural host eggs were dark and nearly completely mottled with spots so differentiating spot from background was also impossible by eye.

We found that the spots we painted on model eggs were noticeably different than the spots of host and cowbird eggshell spots in terms of chromatic (model to host: 1.25±0.07 JND; model to cowbird: 1.44±0.07 JND; figure S2) and achromatic contrast (model to host: 8.33±0.72 JND; model to cowbird: 9.86±0.52 JND; figure S2). Although the avian perceived colour differences between the experimental egg spots and natural mockingbird eggshell spots were relatively low, these were significantly greater than chromatic and achromatic contrasts between host and cowbird spots (0.30±0.10, and 4.96±0.74 JND, respectively; figure S2, Table 3). The chromatic and achromatic contrasts between the spots we painted on experimental eggs and natural cowbird eggs were similar (Table 3, figure S2).

Supplementary Figures

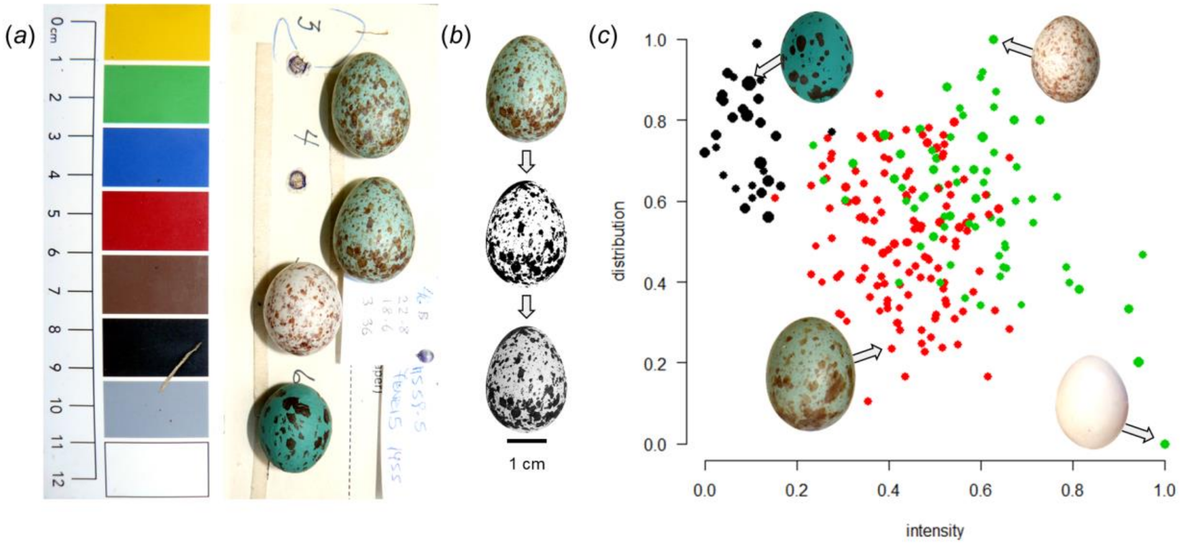

Figure S1. Clutches were (*a*) photographed against a standardized background in the field, and (*b*) individual eggs were isolated and transformed into binary images to identify spots, and their intensities were then determined by subtracting their original image from their detected spots (see electronic supplementary material for more details). The scale applies to all inset egg photographs. We plot the (*c*) normalized distribution of spots (0 = spots entirely found on one pole; 1 = spots evenly distributed across the egg), against their normalized intensity (0 = black, 1 = white), where dot size indicates relative spot size. The colour of the dots represents the type of egg (hosts = red; cowbird = green, and experimental egg model = black). Eggs in the bottom right hand corner have sparse light spots (an example pure white shiny cowbird egg plotted for reference), while those in the bottom left hand corner have sparse darker spots aggregated toward a pole (a host egg plotted for reference). Eggs at the top of the plot have well distributed spots (i.e., relatively consistent distances between spots), we illustrate an example of evenly distributed dark spots (experimental egg model at top left of plot) and evenly distributed lighter spots (parasite egg at top right of plot).

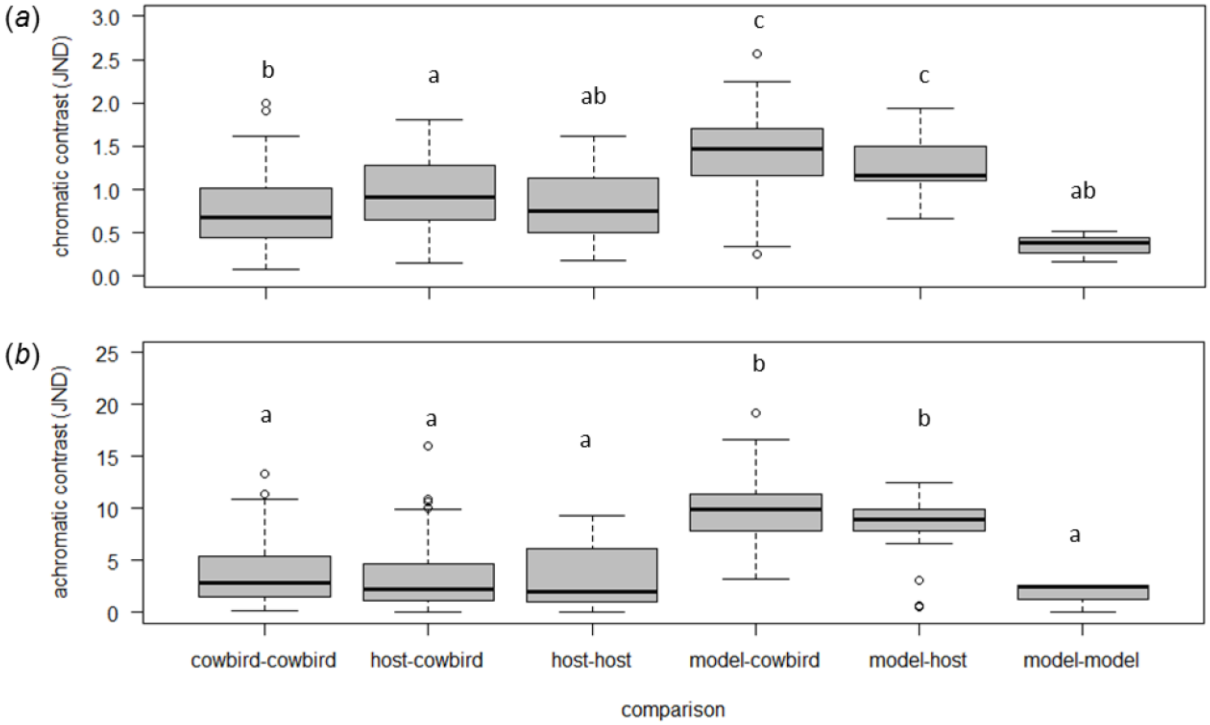

Figure S2. Here we illustrate (*a*) chromatic and (*b*) achromatic contrasts among spots on cowbird, host, and experimental egg models. The letters above the boxes represent Tukey honest significant differences.

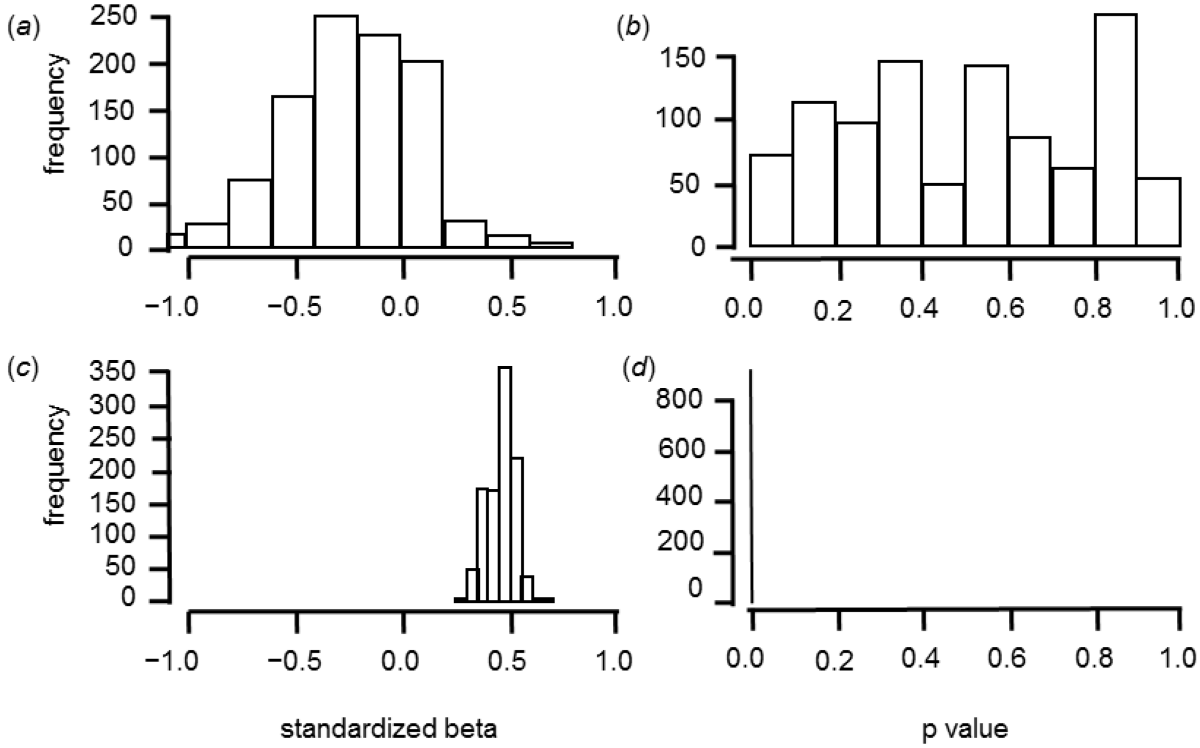

Figure S3. Histograms depicting the model output from binomial generalized linear models predicting host response by (*a*-*b*) achromatic contrast and (*c*-*d*) directional colour differences. We depict standardize effect estimates (*a*, *c*) and p-values (*b*, *d*) from 1,000 analyses based on random subsets of our entire dataset (n = 35 of 70). To aid comparisons, the ranges for standardized effects are depicted from −1 to 1, and for significances are depicted from 0 to 1. The significances for models including directional colour differences as a potential predictor were all < 0.001, and therefore appear as a line.

Supplementary Tables

Table S1. The results from an analysis of variance comparing chromatic and achromatic contrasts of spots between six distinct comparisons: spots among cowbird, host, and model eggs; spots between hosts and cowbird and model eggs; and spots between model and cowbird eggs. Here we report whole model statistics, unstandardized estimates and their standard errors (s.e., both in JND units). Here, parameters are contrast against the differences between host and cowbird eggs (not shown), for a visualization of all differences and their post-hoc significances please refer to figure S2. Significant models and effects are italicized.

	Parameter	b	s.e	t	*p*-value
chromatic contrast ($R^2 = 0.29$, $F_{5,270} = 21.6$, $P < 0.0001$)					
	Intercept	*0.95*	*0.04*	*23.63*	*< 0.0001*
comparison	*cowbird to cowbird*	*−0.20*	*0.06*	*−3.48*	*0.0006*
	host to host	−0.15	0.10	−1.57	0.12
	model to cowbird	*0.49*	*0.07*	*6.64*	*< 0.0001*
	model to host	*0.30*	*0.10*	*3.18*	*0.002*
	model to model	*−0.60*	*0.23*	*−2.56*	*0.01*
achromatic contrast ($R^2 = 0.41$, $F_{5,270} = 36.8$, $P < 0.0001$)					
	Intercept	*3.37*	*0.31*	*10.89*	*<0.0001*
comparison	cowbird to cowbird	0.28	0.45	0.62	0.53
	host to host	−0.10	0.74	−0.13	0.89
	model to cowbird	*6.48*	*0.57*	*11.45*	*< 0.0001*
	model to host	*4.96*	*0.74*	*6.72*	*< 0.0001*
	model to model	−1.65	1.80	−0.92	0.36

Table S2. The Tukey honest significant differences (Tukey HSD) from an analysis of variances predicting the spot features (size, distribution, intensity) by the type of comparison: mockingbird eggs to experimental, cowbird eggs to experimental, cowbird to mockingbird. Data were selected based on a subsampling procedure where we selected (when available) a single host, parasite, and experimental egg from each nest, which was repeated 100 times (see above). This process resulted in 100 tables of Tukey HSD results, thus all statistical parameters (even the p-values) we present as mean±s.e..

Spot features		**difference**	**LCL**	**UCL**	***p*-value**
Size	mockingbird-experimental	−0.25±0.0003	−0.31±0.0003	−0.19±0.0004	all $p < 0.0001$
	cowbird-experimental	−0.20±0.0006	−0.26±0.0006	−0.14±0.0006	all $p < 0.0001$
	cowbird-mockingbird	0.05±0.0007	−0.005±0.0006	0.10±0.0007	0.10±0.006
Distribution	mockingbird-experimental	−0.28±0.003	−0.37±0.003	−0.19±0.002	all $p < 0.01$
	cowbird-experimental	−0.17±0.002	−0.26±0.002	−0.07±0.002	all $p < 0.0001$
	cowbird-mockingbird	0.11±0.002	0.03±0.002	0.19±0.003	0.01±0.002
Intensity	mockingbird-experimental	0.34±0.001	0.28±0.001	0.4±0.001	all $p < 0.0001$
	cowbird-experimental	0.50±0.001	0.44±0.001	0.57±0.001	all $p < 0.0001$
	cowbird-mockingbird	0.16±0.001	0.11±0.001	0.22±0.001	all $p < 0.0001$

Table S3. The mean ± s.e. percentage of correct classifications of eggs to three distinct classes: experimental egg models (experimental), mockingbird eggs (mockingbird), or natural cowbird eggs (cowbird). Values were derived from 100 separate analyses (see methods).

		true state		
predicted state	egg	experimental	mockingbird	cowbird
	experimental	95.52±0.53	0.34±0.34	-
	mockingbird	2.10±0.25	81.94±0.92	21.61±0.55
	cowbird	-	24.67±1.17	70.22±0.76

Table S4. Here we illustrate the backward step-wise elimination procedure used to arrive at a single generalized linear models predicting the probability of mockingbirds rejecting foreign egg models. We begin by using a global model with all the variables of interest (see Methods), then subsequently, we illustrate all full model statistics and all parameter estimates for every step in the process until a final reduced model is achieved. Here “Chromatic contrast” and “achromatic contrast” illustrate the difference in perceivable coloration and luminance between egg models and the average mockingbird egg, respectively (see Methods for more details). “Directional colour” represents the change in log-odds of rejecting egg models that are one just noticeable difference (JND) more blue-green (negative) or browner (positive) than the average mockingbird egg. “Spot” represents the presence or absence of spotting and is coded as unspotted (0) or spotted (1). “Cowbird eggs” and “mockingbird eggs”, represent the number of cowbird and mockingbird eggs (respectively) at the time of experimentation. Flush represents whether the mockingbird was flushed from the nest (1) or not (0). Nest age represents the age of the nest, relative to clutch completion, at the time of the experiment (in days), while date represents the time of the experiment (ordinal days). For each whole model we present Nagelkerke’s R^2 and AIC_c. For all parameters, we present estimates and their associated their standard errors (s.e.), the lower and upper limits of the 95% confidence interval (LCL and UCL), a z-score, and variance inflation factors (VIF). The parameters themselves are identical to table 1 from the main text (see for more details). Significant models and effects are italicized.

predictor	**estimate**	**s.e.**	**LCL**	**UCL**	***z***	**χ^2**	**d.f.**	***p*-value**	**VIF**
full model (χ^2= 24.89, R^2 = 0.40, AIC_c = 95.36, p = 0.003)									
Intercept	0.55	0.45	−0.30	1.50	1.23	–	1	0.22	–
chromatic contrast	0.08	0.41	−0.73	0.91	0.18	0.03	1	0.85	2.04
directional colour	1.00	0.59	−0.07	2.26	1.71	3.36	1	0.07	3.73
spot	*−1.33*	*0.63*	*−2.64*	*−0.15*	*−2.12*	*4.89*	*1*	*0.03*	*1.17*
directional colour *spot	−0.44	0.69	−1.87	0.89	−0.63	0.41	1	0.52	2.49
cowbird eggs	*−0.82*	*0.38*	*−1.61*	*−0.11*	*−2.19*	*5.11*	*1*	*0.02*	*1.57*
mockingbird eggs	0.10	0.35	−0.60	0.82	0.29	0.09	1	0.77	1.40

predictor	**estimate**	**s.e.**	**LCL**	**UCL**	***z***	**χ^2**	**d.f.**	***p*-value**	**VIF**
flush	−0.50	1.22	−3.04	1.88	−0.41	0.17	1	0.68	1.26
date	−0.49	0.39	−1.28	0.26	−1.27	1.66	1	0.20	1.64
nest age	0.31	0.33	−0.33	0.97	0.95	0.91	1	0.34	1.22
first step (χ^2= 24.86, R^2 = 0.40, AIC_c = 92.67, p = 0.002)									
Intercept	0.54	0.44	−0.30	1.47	1.22	–	1	0.22	–
directional colour	*0.93*	*0.45*	*0.11*	*1.91*	*2.08*	*4.99*	*1*	*0.03*	*2.18*
spot	*−1.32*	*0.62*	*−2.61*	*−0.14*	*−2.12*	*4.86*	*1*	*0.03*	*1.15*
directional colour *spot	−0.40	0.66	−1.78	0.87	−0.61	0.38	1	0.54	2.29
cowbird eggs	*−0.81*	*0.37*	*−1.59*	*−0.11*	*−2.18*	*5.09*	*1*	*0.02*	*1.54*
mockingbird eggs	0.11	0.35	−0.60	0.82	0.30	0.09	1	0.77	1.40
flush	–0.55	1.19	−3.03	1.80	−0.46	0.22	1	0.64	1.19
date	−0.52	0.35	−1.24	0.15	−1.50	2.33	1	0.13	1.33
nest age	0.31	0.33	−0.33	0.97	0.95	0.91	1	0.34	1.22
second step (χ^2= 24.77, R^2 = 0.40, AIC_c = 90.12, p < 0.001)									
Intercept	0.52	0.44	–0.31	1.43	1.19	–	1	0.23	–

predictor	**estimate**	**s.e.**	**LCL**	**UCL**	***z***	χ^2	**d.f.**	***p*-value**	**VIF**
directional colour	*0.92*	*0.44*	*0.10*	*1.88*	*2.07*	*4.91*	*1*	*0.03*	*2.13*
spot	*–1.28*	*0.61*	*–2.53*	*–0.13*	*–2.11*	*4.79*	*1*	*0.03*	*1.09*
directional colour *spot	–0.36	0.65	–1.70	0.89	–0.56	0.32	1	0.57	2.18
cowbird eggs	*–0.85*	*0.35*	*–1.59*	*–0.22*	*–2.47*	*7.19*	*1*	*<0.01*	*1.35*
flush	–0.51	1.19	–2.99	1.83	–0.43	0.19	1	0.66	1.18
date	–0.50	0.34	–1.20	0.15	–1.48	2.25	1	0.13	1.27
nest age	0.32	0.32	–0.31	0.98	0.98	0.98	1	0.32	1.20
third step (χ^2= 24.58, R^2 = 0.40, AIC_c = 87.75, $p < 0.001$)									
Intercept	0.47	0.42	−0.33	1.34	1.12	–	1	0.26	–
directional colour	*0.92*	*0.44*	*0.11*	*1.88*	*2.09*	*5.02*	*1*	*0.03*	*2.14*
spot	*−1.27*	*0.60*	*−2.52*	*−0.13*	*−2.11*	*4.79*	*1*	*0.03*	*1.09*
directional colour *spot	−0.32	0.63	−1.62	0.91	−0.50	0.25	1	0.62	2.13
cowbird eggs	*−0.86*	*0.35*	*−1.60*	*−0.22*	*−2.48*	*7.29*	*1*	*< 0.01*	*1.35*
date	−0.45	0.32	−1.10	0.17	−1.43	2.06	1	0.15	1.13
nest age	0.33	0.32	−0.30	0.98	1.01	1.04	1	0.31	1.20
fourth step (χ^2= 24.33, R^2 = 0.39, AIC_c = 85.53, $p < 0.001$)									

predictor	**estimate**	**s.e.**	**LCL**	**UCL**	z	χ^2	**d.f.**	p**-value**	**VIF**
Intercept	0.44	0.40	−0.34	1.26	1.09	–	1	0.28	–
directional colour	*0.77*	*0.31*	*0.18*	*1.42*	*2.47*	*6.57*	*1*	*0.01*	*1.09*
spot	*−1.26*	*0.60*	*−2.52*	*−0.12*	*−2.09*	*4.70*	*1*	*0.03*	*1.08*
cowbird eggs	*−0.82*	*0.33*	*−1.53*	*−0.21*	*−2.48*	*7.07*	*1*	*<0.01*	*1.26*
date	−0.43	0.31	−1.07	0.18	−1.38	1.92	1	0.17	1.10
nest age	0.32	0.32	−0.31	0.97	0.99	0.99	1	0.32	1.19
fifth step ($\chi^2 = 23.34$, $R^2 = 0.38$, $AIC_c = 84.12$, $p < 0.001$)									
Intercept	0.40	0.39	−0.37	1.20	1.01	–	1	0.31	–
directional colour	*0.80*	*0.31*	*0.22*	*1.44*	*2.59*	*7.30*	*1*	*<0.01*	*1.08*
spot	*−1.20*	*0.59*	*−2.42*	*−0.08*	*−2.03*	*4.38*	*1*	*0.04*	*1.05*
cowbird eggs	*−0.71*	*0.31*	*−1.36*	*−0.14*	*−2.32*	*6.09*	*1*	*0.01*	*1.08*
date	−0.44	0.31	−1.07	0.17	−1.41	2.00	1	0.16	1.11
reduced model ($\chi^2 = 21.34$, $R^2 = 0.35$, $AIC_c = 83.80$, $p < 0.0001$)									
Intercept	0.43	0.39	−0.33	1.22	1.09		1	0.28	–
directional colour	*0.91*	*0.30*	*0.36*	*1.53*	*3.07*	*10.76*	*1*	*< 0.01*	*1.04*
spot	*−1.31*	*0.58*	*−2.51*	*−0.21*	*−2.25*	*5.46*	*1*	*0.02*	*1.05*

predictor	**estimate**	**s.e.**	**LCL**	**UCL**	***z***	χ^2	**d.f.**	***p*-value**	**VIF**
cowbird eggs	*−0.64*	*0.29*	*−1.25*	*−0.09*	*−2.20*	*5.26*	*1*	*0.02*	*1.03*

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