

COLORFUL EGGS AND PLUMAGE IN BIRDS:
ECOLOGICAL DRIVERS OF COLOR PATTERNS FILTERED THROUGH BIRD
VISUAL SYSTEMS

BY

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DISSERTATION

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ABSTRACT

Birds use color signals for multiple functions, including attracting and choosing mates, recognizing their own offspring from those of unrelated conspecifics or obligate brood parasites, foraging for different food resources, or hiding from predators. Importantly, an integrative approach is necessary to effectively address how and why birds have evolved to perceive and produce extravagant colors and patterns. Color signal information content, color perception, and cognitive decision rules underlying behaviors in response to color are equally important. The goal of my doctoral dissertation is to understand how birds use colorful signals, including colorful eggs and feathers, to make decisions that affect their fitness. Chapter 1 discusses the implications of categorical perception of color in animals and emphasizes the importance of ecological context in shaping how animals categorize stimuli. Chapter 2 thoroughly tests American robin (*Turdus migratorius*) perception and categorization of egg color, egg size, and egg pattern features when deciding whether to accept or reject a foreign egg present in the nest. Chapter 3 experimentally tests how American robin color perception and visual acuity integrate when robins view and decide to reject foreign eggs from their nests. Chapter 4 investigates how brood parasite host species phenotypes, shaped by foraging ecology, may constrain or facilitate host species ability to recognize and reject brood parasite eggs from their nests. Chapter 5 assesses how ecological pressures reducing the time available and increasing the effort necessary to

find a compatible mate may promote the evolution of plumage sexual dichromatism in true thrushes (*Turdus* spp.). Overall, results from this dissertation demonstrate that, respectively, colorful eggs and plumage likely serve to facilitate recognition of offspring and mates.

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CHAPTER 1: TESTING CATEGORICAL PERCEPTION WITH AN EMPHASIS ON ECOLOGICAL CONTEXTS AND FITNESS COSTS: A COMMENT ON GREEN ET AL. (2020) ¹

Context-Dependent Categorical Perception

In their timely and detailed perspective piece, Green et al. (2020) propose diverse decision-making contexts where extensive overlap between continuous stimuli, limited timeframe of perception-to-decision latency, and high fitness costs of not responding, or committing recognition errors, all may favor the evolution and development of categorical perception. We agree with the predictions made by Green et al. (2020) and emphasize the need for careful selection of the experimental contexts in which categorical perception is to be tested. Notably, amongst the many examples cited as evidence of categorical perception across animals in Green et al. (2020), only two examples involved studying animals outside the lab in the wild (Swamp Sparrows *Melospiza georgiana*) or in semi-natural habitats (Japanese macaques *Macaca fuscata*). Therefore, first and foremost, when feasible, future experiments should be conducted in the natural habitat of the species and individual(s) of interest (or, at least, closely resemble the natural contexts that the subjects experience), and during time periods

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when swift decision-making, guided by categorical perception, may be advantageous for survival and/or reproductive success.

For example, a useful paradigm for field-based experiments highlighted by Green et al. (2020) is egg rejection by hosts of avian brood parasites, where parasitized individuals must recognize and remove (often mimetic) brood parasitic eggs laid in their nest to avoid the clearly-measurable fitness costs associated with raising unrelated offspring (Hauber 2003). Categorical perception in this system can be readily tested by placing foreign eggs which vary along a continuous color and/or pattern gradient into host nests and monitoring egg rejection responses (e.g., Hanley et al. 2017).

Additionally, categorical perception may facilitate mate recognition and choice in species that search for suitable mates amongst signals and cues produced by both conspecifics and heterospecifics during a limited time period. For example, co-occurring light-signaling *Photinus* firefly species must locate conspecific mates while avoiding signals produced by both incompatible congeners and predators capable of mimicking flash signals (Lewis and Cratsley 2008).

Moreover, the design and presentation of experimental stimuli and the behavioral responses measured, along with socio-ecological context tested, are equally important when testing for categorical perception. Stimuli should be designed with continuous variation along an axis that is both representative of the natural variation to

which subjects are exposed to in the wild and in perceivable units to the species of interest (e.g., “just-noticeable differences,” JNDs), because sensory systems often process differences between stimuli on a proportional rather than absolute scale (Akre and Johnsen 2016). Finally, measured behavioral responses should be indicative of discrimination or choice, while also accounting for focal animal responsiveness and random response outcomes.

Linking Fitness Costs To (Lack Of) Categorical Perception

For categorical perception to be a valuable concept in behavioral ecology, it must have a measurable connection to or effect on fitness. Specifically, there could be 1) significant fitness outcomes (i.e., costs or benefits) associated with the perception of, assessment of, and the decision-making based upon continuously scaled stimuli, and 2) measurable avoidance or mitigation of fitness costs through categorization of the stimuli when making decisions. Determining the type and extent of fitness costs associated with a lack of categorical perception should be a fundamental goal, albeit a challenging one. Social recognition tasks, for instance, may be exceptionally useful for determining both the presence of and fitness costs associated with categorical perception because of their well-documented consequences for reproductive decisions and subsequent fitness. Accordingly, recognition errors are often made when recognizing offspring, kin, and/or compatible mates (Hepper 1991; Rosenthal 2017),

resulting in the rejection of one's own offspring (Davies et al. 1996) or mating with a relative or heterospecific (Rosenthal 2017). For instance, categorical perception may be more likely to facilitate mate preferences in sympatric rather than allopatric heterospecific populations and underlie the evolution of conspicuous reproductive character displacement that reduces receiver mate recognition errors (and categorization errors) and subsequent introgression and hybridization (Akre and Johnsen 2016). Mate recognition experiments with heterospecific individuals from both sympatric and allopatric populations could test the prediction that sympatric individuals, but not allopatric individuals, should have stringent categorical perception of continuous signals used for assessing potential mates.

Conclusions

Categorical perception is a timely topic worth exploring for behavioral ecologists, and Green et al. (2020) provide a current perspective of its history, recent developments, and potential future directions. We emphasize the necessity of testing for categorical perception in study systems and contexts where its effects on fitness can be estimated or measured. Categorical perception may prove to be widespread among animals, and behavioral ecologists have a unique opportunity to place categorical perception in an ecological and evolutionary context.

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CHAPTER 2: WHICH EGG FEATURES PREDICT EGG REJECTION RESPONSES IN AMERICAN ROBINS? REPLICATING ROTHSTEIN'S (1982) STUDY ²

Abstract

Rothstein (1982) was one of the first comprehensive studies to examine how different egg features influence egg rejection behaviors of avian brood parasite hosts. The methods and conclusions of Rothstein (1982) laid the foundation for subsequent experimental brood parasitism studies over the past thirty years, but its results have never been evaluated with replication. Here, we partially replicated Rothstein's (1982) experiments using parallel artificial model egg treatments to simulate cowbird (*Molothrus ater*) parasitism in American robin (*Turdus migratorius*) nests. We compared our data with those of Rothstein (1982) and confirmed most of its original findings: (i.) robins reject model eggs that differ from the appearance of a natural robin egg toward that of a natural cowbird egg in background color, size, and maculation, (ii.) rejection responses were best predicted by model egg background color, and (iii.) model eggs differing by two or more features from natural robin eggs were more likely to be rejected than model eggs differing by one feature alone. In contrast with Rothstein's

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(1982) conclusion that American robin egg recognition is not specifically tuned toward rejection of brown-headed cowbird eggs, we argue that our results and those of other recent studies of robin egg rejection suggest a discrimination bias toward rejection of cowbird eggs. Future work on egg recognition will benefit from utilizing a range of model eggs varying continuously in background color, maculation patterning, and size in combination with avian visual modelling, rather than using model eggs which vary only discretely.

Introduction

Reproducibility is a central concern of the modern scientific approach and paramount to research progress (Kelly 2006; Nakagawa and Parker 2015; Baker 2016). Confidence in empirical conclusions depends on successful replication of experimental data and results; that is, if a discoverable general pattern exists in nature, then it should be measurable, consistent, and reproducible by multiple independent laboratories (Simons 2014). Here, we reevaluate critical and impactful findings from one of the first comprehensive studies on brood parasite egg rejection by avian hosts that examined how different features of foreign eggs influence host rejection decisions.

Interspecific avian brood parasites do not build their own nests or raise their own offspring; instead, they lay their eggs in the nests of host species (Davies 2000). Hosts may accept the brood parasite's egg and raise unrelated offspring at a cost to their own

fitness (Hauber 2003), or recognize the foreign egg and remove it from the nest (Payne 1977). Stephen Rothstein's dissertation at Yale University, the many resulting papers, and especially his landmark *Behavioral Ecology and Sociobiology* 1982 study of egg rejection by American robins (*Turdus migratorius*; hereafter: robin) and gray catbirds (*Dumetella carolinensis*) paved the way for subsequent studies focusing on characterizing robin and other host species' abilities to discriminate their own eggs from those of their respective brood parasites (or from foreign eggs in general; Hauber et al. 2015).

Through the careful design of artificial model eggs constructed from plaster of Paris and painted with acrylic and latex paints, Rothstein (1982) separated the relative influences of egg size, background color, and spotting (or "maculation") on robin and gray catbird egg rejection responses. Egg rejection behavior in robins likely evolved as a defense against brood parasitism by the mostly sympatric brown-headed cowbird (*Molothrus ater*; hereafter: cowbird), an obligate interspecific brood parasite (Friedmann 1929; Rothstein 1975a; Briskie et al. 1992; Croston and Hauber 2014; Lang et al. 2014; Kuehn et al. 2014; Abernathy and Peer 2015; Croston and Hauber 2015a). Notably, Rothstein (1982) found that robins respond most strongly to experimental model eggs that deviate from their own eggs' appearance and toward that of a cowbird egg in at least two of the three features tested (i.e., background color, maculation and size), and

respond only weakly to experimental eggs that differ from their own eggs by one feature alone.

Here, we set out to replicate Rothstein's (1982) experiments using a parallel set of artificial model eggs to reexamine the relative influence of discrete differences in model egg background color, maculation, and size on robin egg rejection decisions. We conducted a partial replication (see Kelly 2006; Nakagawa and Parker 2015) of Rothstein's (1982) experimental methods, combined data from our experiments with those of Rothstein (1982), and analyzed which egg features predict robin egg rejection responses with an information-theoretic statistical approach using generalized linear mixed models (Burnham and Anderson 2002; Bolker et al. 2009; Symonds and Moussalli 2011).

Methods

Data and model eggs

We extracted the published model-type level egg rejection data from Rothstein (1982) of robins' responses to various experimental eggs placed into their nests (for data source: see Rothstein 1982's Fig. 2) to combine with our own data. Artificial model eggs of both studies were specifically designed to represent a discrete spectrum of egg sizes, background colors, and maculation pattern combinations ranging in appearance from a robin egg to a cowbird egg (Fig. 2.1). We re-coded and binned model egg data from

Rothstein (1982) as follows (original coding indicated by $R'82$ subscript): $W_{(R'82)}$ = white/beige, cowbird-mimetic background color; $S_{(R'82)}$ = small, cowbird-sized; $M_{(R'82)}$ and $P_{(R'82)}$ = maculated, mimetic cowbird-colored spotting. Experimental model eggs' features of both Rothstein (1982) and our study are regarded as follows: *size* = robin-sized (L) or cowbird-sized (S); *background color* = robin blue-green colored (M) or cowbird white/beige colored (B); *maculation* = spotted (S) or immaculate (absence of S code). Model eggs of both Rothstein (1982) and this study were constructed with plaster of Paris and colored using acrylic or latex paints; for painting, size, and manufacture details of our model eggs, see Croston and Hauber (2014), "BHCO ground" and "AMRO (mimetic) ground" colors.

Small cowbird-sized and large robin-sized model eggs used by the current study resembled the dimensions and masses of natural cowbird (2.6–3.4 g, 21×16 mm) and robin eggs (4.2–8.4 g, 31×21 mm). Model eggs from Rothstein (1982) also resembled the dimensions of natural cowbird and robin eggs but were estimated to be 10-17% heavier than natural eggs (Rothstein 1975b). Model egg mass can significantly affect host egg rejection responses, because heavier eggs are less likely to be successfully rejected (Ruiz-Raya et al. 2015). However, robins are adept grasp-ejectors (Rasmussen et al. 2009; Rasmussen et al. 2010), capable of removing model eggs within the range of natural robin egg size and mass (Underwood and Sealy 2006; Rasmussen et al. 2009). The

European blackbird (*Turdus merula*), a congener of American robins with similar body size and bill morphology, can remove model eggs weighing at least 10 grams (Ruiz-Raya et al. 2015). Therefore, it is unlikely that a potential disparity between masses of model eggs used in our experiments versus Rothstein's (1982) would significantly affect the results.

Subjects and study areas

Robins are a rejecter host species of cowbirds (Rothstein 1975a); robin populations sympatric with cowbirds reject about 100% of natural cowbird eggs that are deposited into their nests (Briskie et al. 1992). Rothstein (1982) tested nesting robins in Connecticut and Michigan, USA between the years of 1966-70 (N=93 total parasitism trials). We conducted a total of N=125 experimental parasitism trials in Ithaca, NY from 2010-2014 (N=109) and Urbana, IL in 2015 (N=16). Although there may be some variation in egg rejection behavior between American robin populations that are allopatric vs. sympatric with cowbirds (Briskie et al. 1992), experimental brood parasitism data from all populations of robins studied by Rothstein (1982) and the current study are comparable because cowbirds have been sympatric with robins across all locations where artificial parasitism experiments took place since the U.S Geological Survey began its North American Breeding Bird Survey in 1966 (Sauer et al. n.d.).

Experimental parasitism

For Rothstein's (1982) data, we combined the data of robin responses to model eggs in both the nesting (i.e., laying) and incubation stages (i.e., stages 1 and 2, respectively) because our data also came from both nesting periods unseparated. The nest stage at either the start or end of an experimental parasitism trial does not significantly influence robins' responses to the model eggs placed in their nests at the Ithaca and Urbana study sites where our own experiments took place (Croston and Hauber 2014; Luro and Hauber 2017). Likewise, Rothstein (1982) acknowledged results would have only been slightly different if data from both nesting stages were combined.

For our own experiments, we followed the experimental brood parasitism methods of Igic et al. (2015). In brief, a model egg was placed into an active robin nest (i.e., nest containing one or more eggs) found in either the laying or incubating stage. Unlike Rothstein (1982), we did not remove a single robin egg from the nest and replace it with an experimental model egg, because the removal of natural robin eggs from the nest does not affect robin responses to model eggs placed in the nest (Briskie et al. 1992). Furthermore, cowbirds may not always remove a host egg before or after parasitizing a nest (Scott 1977; Sealy 1992). Therefore, the insertion of a foreign egg into a robin's nest alone, without removing a robin's own egg, is sufficient to simulate natural cowbird parasitism for this host species.

For both our and Rothstein's (1982) data, responses to model eggs were recorded as rejections if the model egg disappeared from the nest within 5 days from the day it was inserted into the nest. If the model egg remained in the nest after 5 days, the robin's response was recorded as an acceptance. If the nest was deemed abandoned (or deserted), eggs in the nest hatched, or the nest was depredated during the experimental period, the experimental trial was ended and excluded from the analyses. For a more detailed explanation of our experimental parasitism procedures on robins using plaster of Paris model eggs, see Croston and Hauber (2014) and Aidala et al. (2015). Critically, these studies found no effect of repeated parasitism and nesting stage (laying vs. incubation) on egg rejection rates by American robins. Data from our own artificial parasitism experiments could not be collected blindly because our study involved observation of wild robin nests in the field.

Statistical analyses

To assess the relative influences of experimental egg size, background color and maculation on robin egg rejection responses, we used generalized linear mixed models (GLMMs), constructed with the *glmer* function and fitted with Laplace approximations and binomial logit distributions using the *lme4* package (Bates et al. 2015) in combination with model averaging. All analyses were conducted in R v.3.2.4 (R Core Team 2017).

Initially, to assess if combining of our own data with Rothstein's (1982) would be appropriate, we first analyzed a set of GLMMs using the MuMIn package with study ID (i.e., Rothstein 1982 or this study's experimental data), along with model egg features and of all their interactions with study as fixed effects. Then, we selected the best models (see below) and calculated model averaged effect estimates and their 95% confidence intervals for all predictors included in the top models (the procedure for best model selection and model average effect estimates are explained below).

After finding no significant differences between Rothstein's (1982) and our own data (see Results), we then analyzed a new set of GLMMs which accounted for variation in robin rejection behaviors across the two studies by setting study ID as a random effect. For our global GLMM, the binary response variable was the rejection/acceptance of the artificial model egg, random effects included nest ID and study ID, and fixed effects included the model egg color (background blue-green robin or white-beige cowbird), maculation (spotted or immaculate), size (robin-sized or cowbird-sized) and the interactions among all three model egg features. We ran all possible combinations of predictors included in the global GLMM as model iterations using the dredge function from the MuMIn package and selected the best models using Akaike's information criteria corrected for sample size (AICc), setting a cutoff to include the fewest number of models whose sum of Akaike weights (w_i) is greater than $w_i = 0.95$ (Burnham and

Anderson 2002; Wagenmakers and Farrell 2004). Finally, we calculated model-averaged effect estimates and 95% confidence intervals for all predictors included in the three final best models (Table 2.1).

Results

Comparison between studies

We did not find a significant difference in robin rejection responses between Rothstein (1982) and our own experiments (Rothstein (1982) vs. current study log odds: -0.65, 95 % CI= [-1.44, 0.15]). We also did not find any significant effects of interactions between study ID and the size, background color, or maculation pattern of the artificial model eggs (all research time interaction variables' model-averaged effect estimate 95% CIs overlapped 0). Therefore, we accounted for variance in robin responses attributable to study ID by including study ID as a random effect in our final set of GLMMs.

Rejection responses toward specific model egg treatments

Model eggs with features resembling those of natural brown-headed cowbird eggs (beige background color, small size and maculation) were rejected at higher rates than model eggs with features resembling those of natural robin eggs (blue-green background color, large size, and immaculate) (Fig. 2.2). Robin egg rejection responses were best predicted by model egg background color (beige vs. blue-green log-odds: -3.80, 95 % C.I. = [-5.62, -1.98]) and maculation (immaculate vs. spotted log-odds: 3.4, 95

% C.I. = [1.66, 5.15]), but also by model egg size (large vs. small log-odds: 2.23, 95 % C.I. = [0.70, 3.77]) (Tables 2.1, 2.2). The effect of robin (large) vs. cowbird-sized (small) eggs was different for beige vs. blue eggs (interaction between egg size and background color, log-odds: -3.33, 95 % C.I. = [-5.89, -0.77]; Fig. 2.2). The effect of model egg maculation on rejection probability did not vary between the two egg sizes (interaction between egg size and maculation, log-odds: -0.45, 95 % C.I. = [-3.60, 0.91]) nor between egg background colors (interaction between egg background color and maculation, log-odds: 0.12, 95 % C.I. = [-1.7, -2.95]) (Tables 2.1, 2.2).

Discussion

American robins' responses to egg background color, maculation, and size

Similar to Rothstein's (1982) results, our study found that American robins responded to model egg features of background color, size, and maculation, and were more likely to reject a model egg when it deviated from the natural robin egg appearance toward the natural cowbird egg appearance by at least two features (Table 2.2 and Fig. 2.2). Results from the combined dataset of the two studies also confirmed that robin rejection decisions are best predicted by model egg background color and maculation, relative to model egg size. Additionally, model egg size and background color together influenced robin egg rejection responses (Tables 2.1, 2.2); consistent with Rothstein's (1982) conclusion that robins may have a "tolerance" for eggs which vary in

color or size alone but will predictably reject model eggs which differ from natural robin egg appearance in both size and color (Fig. 2.2, 2.3). Because natural robin eggs can be quite variable in both size and color (Croston and Hauber 2015b), tolerance of eggs varying in one of these two features alone may reduce possibility of mistakenly rejecting some of their own eggs (Rothstein 1982).

Differences from Rothstein's (1982) findings

However, in contrast to Rothstein's (1982) conclusion that differences in at least two egg features are required to evoke any egg rejection responses in robins, the combined data revealed that robins do indeed recognize and reject foreign eggs that differ from their own eggs by a single feature toward the appearance of a cowbird egg. Specifically, model eggs differing from natural robin egg appearance in background color, maculation, or size alone were rejected at considerable rates in comparison to the complete absence of rejection responses toward completely robin-mimetic control model eggs used for our own experiments (Fig. 2.2, 2.3, *LM*). Maculated model eggs increased rejection to 68% (Fig. 2.2, *LMS*), model eggs with background color resembling cowbird egg color increased rejection to 37% (Fig. 2.2, *LB*), and small cowbird-sized model eggs increased rejection to 16% (Fig. 2.2, *SM*). The greatest difference between robins' responses to model eggs within a replicated treatment used by this study and that of Rothstein (1982) was for large cowbird-colored model eggs: 37

% rejection in this study and 0 % rejection in Rothstein's (1982) (combined data Fig. 2.2, *LB*; see Fig. 2.3 *LB* for rejection response difference between separated study treatments). This disparity is likely due to the different paint colors used by this study and Rothstein (1982) to simulate natural cowbird egg background color, and possibly the smaller sample size ($n=8$) for this model egg treatment in Rothstein (1982) (Fig. 2.3, egg treatment *LB*). Rothstein (1982) used white paint, chosen using Munsell color chips (Munsell 1965), to simulate natural cowbird egg background color, whereas we used beige paint that generally matches natural cowbird egg background reflectance spectra (see Croston and Hauber 2014 "BHCO ground" for details).

Rothstein (1982) originally concluded that robin egg rejection is guided by comparing an internal representation of natural robin eggs (an own egg "template") with foreign eggs, and is not specifically tuned toward rejection of cowbird-like eggs. However, recent work suggests that robins do have substantial specificity in their egg recognition thresholds toward an intolerance of cowbird eggs. Hanley et al. (2017) demonstrated robins' rejection decisions are fine-tuned to the gradient of natural egg colors, but robins ignore perceivable differences along artificial color gradients, a finding inconsistent with the internal "own egg vs. foreign egg" template (or multiple thresholds, sensu Hanley et al. 2017) hypothesis. Similarly, Dainson et al. (2017) also found that robin egg rejection responses to egg spot coloration are likely tuned to a

gradient of natural egg color patterns, where robins are more inclined to reject model eggs that have highly contrasting brown spots against a mimetic blue-green robin egg background color.

In summary, Rothstein's (1982) benchmark study set the standard for research on host recognition of brood parasite eggs. Since its publication, a vast amount of methodologically similar work has investigated which egg features evoke foreign egg rejection behavior in many different avian brood parasite host species (Underwood and Sealy 2006; Moskát et al. 2008; López-de-Hierro and Moreno-Rueda 2010; de la Colina et al. 2012; Croston and Hauber 2014; Segura et al. 2016). Generally, the relative difference between own and foreign egg background coloration seems to be the most important determinant of whether hosts accept or reject foreign eggs (Cassey et al. 2008; Moskát et al. 2008; Spottiswoode and Stevens 2010; Hauber et al. 2015).

Future directions for egg recognition research

Here, we confirmed that discrete, categorical differences in egg background color, maculation, and size are all important cues for foreign egg recognition in robins. Importantly, recent experimental approaches have set a new standard, using model eggs, which vary continuously rather than discretely, along natural gradients of different background colors (Hanley et al. 2017), as well as maculation patterns and contrasts (Dainson et al. 2017), and sizes and shapes (Igic et al. 2015). Egg rejection

studies performed with continuously varying model eggs, in combination with avian visual modelling (Avilés 2008; Cassey et al. 2008; Spottiswoode and Stevens 2010), allow for estimation of perceivable differences to the host species of interest for each model egg feature. Thus, they may provide more robust, meaningful comparisons of the relative influence of each separate egg feature for a given host species' egg recognition threshold than studies using model eggs which vary only discretely. Given the recent and rapid development of 3D-printing to construct model eggs of differing shapes and sizes for use in artificial brood parasitism experiments (Igic et al. 2015), and our knowledge of the avian-visible egg color, maculation, and pigment diversity (Hanley et al. 2015), it is now certainly possible to design such a suite of experiments for other brood parasite host species, like those performed with robins (Hanley et al. 2017; Dainson et al. 2017).

Figures and Tables

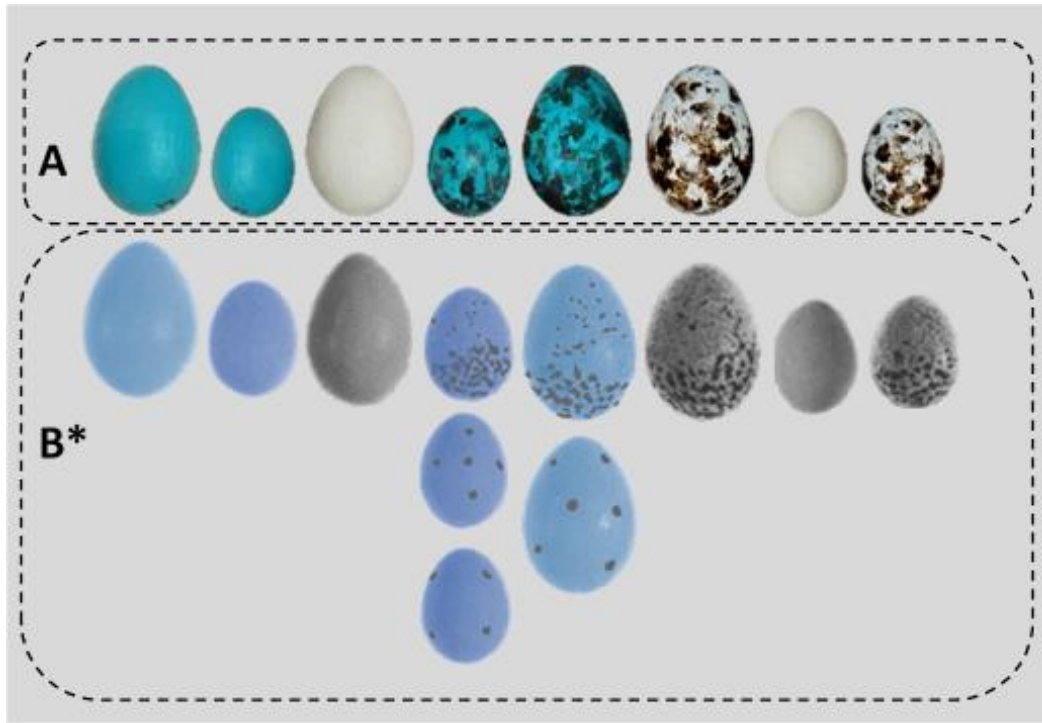


Figure 2.1 Artificial eggs used in parasitism experiments of **A** current study, and **B** Rothstein (1982). Artificial eggs were regarded as the following treatments in analyses, from left to right: large mimetic (*LM*), small mimetic (*SM*), large beige (*LB*), small mimetic spotted (*SMS*), large mimetic spotted (*LMS*), large beige spotted (*LBS*), small beige (*SB*), and small beige spotted (*SBS*). Data for Rothstein's (1982) *SMS* and *LMS* eggs were aggregated for the respective three and two egg treatments shown in **B**. Sizes of eggs used in both studies **A,B** are accurate relative to one another, within each respective study **A,B** *Images of Rothstein's (1982) artificial eggs were adapted from Rothstein's (1982) Fig. 2.1 depiction of model eggs. Images were re-colored for illustrative purposes and do not represent actual color of eggs used in Rothstein (1982).

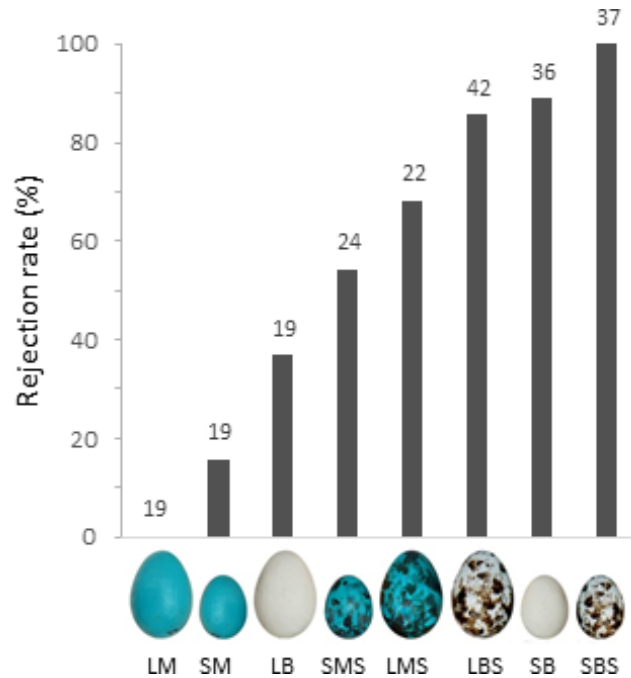


Figure 2.2 Rejection rates of experimental eggs placed in American robin nests. Data used are combined from the current study and Rothstein (1982). Sample sizes of the number of trials for each treatment are listed above bars. Experimental egg treatment codes are in order of size (*L*=large robin-sized, *S*=small cowbird-sized), color (*M*=mimetic robin, *B*=beige cowbird) and maculation (*S*=spotted).

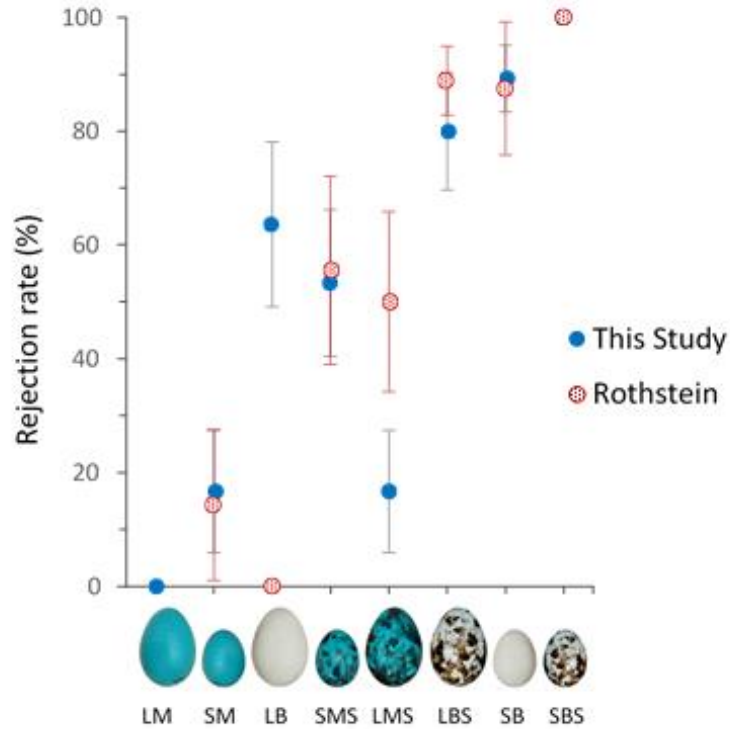


Figure 2.3 Rejection rates of experimental eggs placed in American robin nests separated by study (this study vs. Rothstein 1982). Error bars represent approximate binomial standard error of rejection rates ($\sqrt{\frac{Rejections \cdot 1 - Rejections}{Trials - 1}}$). Experimental egg treatment codes are in order of size (*L*=large robin-sized, *S*=small cowbird-sized), color (*M*=mimetic robin, *B*=beige cowbird) and maculation (*S*=spotted). *LM* treatment done in the current study, but not by Rothstein (1982). Rejection rates for *SBS* treatments of both studies overlap at 100%.

Model 	Model Parameters										df	logLik	AICc	Δi	w_i
	Color	Maculation	Size	Color Maculation	x	Color Size	x	Maculation Size	x	Color Maculation Size					
1	+	+	+			+					7	-83.51	181.55	0	0.48
2	+	+	+			+		+			8	-82.79	182.27	0.72	0.33
3	+	+	+	+		+					8	-83.36	183.40	1.85	0.19

Table 2.1 Model selection table for the three top models from all candidate models (N=16) testing the effects of foreign egg color, maculation, and size on egg rejection decisions by American robins. For all models, Nest ID and Study ID (i.e., this study or Rothstein 1982) were included as random effects. Top models were selected as the models whose cumulative sum of AICc weights (w_i) > 0.95. $\Delta i = AICc(i) - AICc(\min)$.

Model parameter	Model effect estimate (95% CI)	Relative variable importance	Percent of candidate models containing variable
Intercept	1.53 (0.61, 2.45)	---	---
Color (beige → blue-green)	-3.80 (-5.62, -1.98)	1.00	75
Maculation (immaculate → spotted)	3.40 (1.66, 5.15)	1.00	75
Size (large robin → small cowbird)	2.23 (0.70, 3.77)	1.00	75
Color x Size	-3.33 (-5.89, -0.77)	0.88	25
Maculation x Size	-0.45 (-3.60, 0.91)	0.41	25
Color x Maculation	0.12 (-1.70, 2.95)	0.27	25

Table 2.2 Model-averaged mean effect estimates (log-odds) and 95% confidence intervals for the influence of model egg parameters on egg rejection outcomes in American robins from the top three models (see Table 1). Confidence intervals that do not overlap zero are noted in bold. For all models in Table 2.1, Nest ID and Study ID (i.e., this study or Rothstein 1982) were included as random effects.

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CHAPTER 3: VISUAL ACUITY AND EGG SPATIAL CHROMATIC CONTRAST PREDICT EGG REJECTION BEHAVIOR OF AMERICAN ROBINS ³

Abstract

Color and spatial vision are critical for recognition and discrimination tasks affecting fitness, including finding food and mates and recognizing offspring. For example, as a counter defense to avoid the cost of raising the unrelated offspring of obligate interspecific avian brood parasites, many host species routinely view, recognize, and remove the foreign egg(s) from their nests. Recent research has shown that host species visually attend to both chromatic and spatial pattern features of eggs; yet how hosts simultaneously integrate these features together when recognizing eggs remains an open question. Here, we tested egg rejection responses of American robins (*Turdus migratorius*) using a range of 3D-printed model eggs covered with blue and yellow checkered patterns differing in relative square sizes. We predicted that robins would reject a model egg if they could visually resolve the blue and yellow squares as separate features or accept it if the squares blended together and appeared similar in color to the natural blue-green color of robin eggs as perceived by the avian visual

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system. As predicted, the probability of robins rejecting a model egg increased with greater sizes of its blue and yellow squares. Our results suggest that chromatic visual acuity and viewing distance have the potential to limit the ability of a bird to recognize a foreign egg in its nest, thus providing a limitation to host egg recognition that obligate interspecific avian brood parasites may exploit.

Introduction

Animals use spatial chromatic cues when visually detecting predators and prey, choosing mates, and recognizing their own species and close kin (Cuthill et al. 2017; Caves et al. 2018). How animals perceive and discriminate between spatial chromatic visual cues when making decisions relevant to evolutionary fitness is a fundamental question of visual ecology (Cronin et al. 2014; Endler and Mappes 2017). Behavioral experiments of the perception and discrimination of stimuli that vary both chromatically and spatially have been conducted in a variety of animal taxa, including honeybees *Apis mellifera* (Srinivasan and Lehrer 1988; Giurfa et al. 1996), lizards (Fleishman et al. 2017), and birds (Lind and Kelber 2011; Potier et al. 2018). In many of these studies, results from a limited number of captive animals subject to extensive training under controlled laboratory settings are considered to be representative and relevant to the model species' natural behaviors and ecologies. Despite the potential for more direct inference of the effect of visual perception and discrimination on fitness in

the wild, few studies have tested animals' perception, discrimination, and behavioral responses to ecologically and evolutionarily relevant visual stimuli in their natural environments (Endler and Mappes 2017).

Obligate avian brood parasitism is a rare breeding strategy (~1% of all bird species) in which brood parasites lay their eggs in the nests of different species. In response, potential hosts either recognize and reject the foreign egg(s) or nestling(s) or raise the parasitic offspring at a cost to their own fitness (Davies 2000). Visually discriminating own vs. parasitic eggs can be an exceptionally challenging task when brood parasites produce highly mimetic eggs (Stoddard and Hauber 2017). As a result, hosts may commit recognition errors and mistakenly reject their own eggs, rendering egg rejection a risky defense strategy with potentially severe consequences to host fitness (Lotem et al. 1995; Davies et al. 1996).

Arguably, avian brood parasitism research to test how birds recognize eggs is at the forefront of avian visual perception and discrimination experiments conducted in the wild (Stoddard and Hauber 2017). Model plaster (Rothstein 1982; Davies and Brooke 1989; Honza et al. 2007) or 3D printed eggs (Igic et al. 2015) can be systematically painted with ranges of natural avian colors (Hauber et al. 2015; Canniff et al. 2018) and spotting patterns (Dainson et al. 2017; Luro et al. 2018; Hanley et al. 2019) and placed into host species' nests to test if the attending parents discriminate and

recognize the model eggs by touching them (Soler et al. 2017), setting them aside within the nest, or removing them from the nest. Recent work combining visual modeling (Vorobyev and Osorio 1998) with foreign-egg rejection experiments has demonstrated that 1) birds have a perceptual bias towards responding to natural blue-white-brown egg color gradients (Abolins-Abols et al. 2019; Manna et al. 2020) but do not respond predictably to purple-green color gradients not found among natural eggs (Hanley et al. 2017); 2) egg background color and egg spotting presence (and color) combine together as a multicomponent cue and can greatly increase or decrease egg rejection responses, depending on the host species' own egg appearance (Dainson et al. 2017; Luro et al. 2018; Hanley et al. 2019); and 3) host species may use achromatic patterning and spatial features such as egg spotting and scrawling when recognizing and rejecting foreign eggs (Spottiswoode and Stevens 2010; Stoddard et al. 2014). Egg rejection experiments require neither captivity nor extensive training and allow for individually repeated and population-wide tests of avian visual discrimination capabilities in an ecologically relevant context.

Despite great advances in our knowledge of how birds recognize foreign eggs within their nests using visual cues including egg size, shape, color and spotting, no study to date has examined whether egg spatial and chromatic features may be simultaneously integrated by the host's visual system to influence egg recognition. For

instance, how well can birds resolve details of egg spotting pattern and color when viewing eggs from various distances? Do some egg spotting patterns blend in with each other and with the egg background from the bird's point of view? Here, we tested if and how American robins *Turdus migratorius* (hereafter: robins), a robust rejecter of parasitic brown-headed cowbird *Molothrus ater* eggs (Rothstein 1982), respond to differences in spatial chromatic contrast when viewing and deciding to accept or reject a foreign egg.

The underlying assumption is that visual acuity (i.e., visual spatial resolution) can limit the ability of birds to resolve details in the chromatic spatial pattern of eggs, as has been found in diverse taxa under different ecological conditions (Caves et al. 2018). We used avian visual modeling and visual acuity estimates for lateral and binocular vision of American robins to create model eggs with spatial chromatic patterns along a gradient of increasing difficulty for robins to visually resolve the patterned eggs as different from natural robin eggs. To this end, we i) designed a range of 3D-printed model eggs and covered them with a suite of novel, blue and yellow checkered patterns, where both colors were present in equal proportions but differed in their relative inter-square distances to one another across model eggs, and ii) estimated a spatial chromatic discrimination threshold distance at which the blue and yellow squares should blend from the robin's visual perspective and appear similar to the

natural robin egg's immaculate blue-green coloration. We predicted that robins would reject the model egg when they could resolve the blue and yellow squares as separate features, but would accept the artificial egg when they could not resolve the squares apart (i.e., if the blended color of blue and yellow squares appeared similar to the blue-green color of natural robin eggs).

Methods

Experimental egg pattern design

We generated test checkerboard patterns of various combinations of blue and yellow colored squares, whose centers were spaced 0.15 mm apart from one another and printed the test color patterns on transferrable decal paper (Sunnyscopa Film-Free Waterslide Decal Paper Multi Use 8.5x10in [216x254mm], Sunnyscopa, South Korea) using an HP Color LaserJet Pro MFP M281fdw with HP202A black, yellow, cyan and magenta toners (HP Development Company, L.P. USA). Our printer had a color printing resolution of up to 600 x 600 dpi, and we printed patterns at 300 dpi resolution in tagged image file format (TIFF). We took spectral reflectance measurements of the printed test color patterns using an Ocean Optics JAZ spectrometer (Ocean Optics, Inc. USA) with a 400 μ m fiber optic cable and a reflectance probe fitted with a black rubber cap to block external light. We measured a 12.57 mm² circular area of the pattern from a 10 mm distance at 90° angle. The reflectance probe had an acceptance angle of

24.8°, and therefore the reflectance measurements were of both blue and yellow colors together (using Eqn 1.0, 24.8° > 0.015° angle of single 0.150mm blue or yellow square measured from 10mm away).

Eqn 1.0 Swearer (2011)

$$VisualAngle\theta = 2 \cdot atan\left(\frac{ObjectSize}{2 \cdot ViewingDistance}\right)$$

We used a receptor-noise limited model (Vorobyev and Osorio 1998) to calculate avian-perceived chromatic and achromatic Just Noticeable Differences (JNDs) between our “blended” test blue and yellow pattern, the blue color alone, the yellow color alone, and a range of N = 22 natural robin eggs, using reflectance spectra data from Croston and Hauber (2015b) to determine the best printed blue/yellow combination for mimicking natural robin egg colors (Tables 3.1 and 3.2). We used peak sensitivities of UVS, SWS, MWS and LWS cone photoreceptors, cut-off wavelengths (λ_{cut}) for photoreceptors’ respective oil droplets, relative photoreceptor densities, and ocular media transmittance of the congeneric common (European) blackbird *Turdus merula* (Hart et al. 2000). The model assumed robins viewed the eggs under bright daylight conditions (D65 irradiance spectrum from Maia et al. 2019), eggs were viewed against the background of a typical dry brown grass lining in natural robin nests (nest reflectance data were sourced from Aidala et al. 2015), and a Weber fraction value of 0.1 for calculating photoreceptor noise values (Olsson et al. 2018).

Previous work has shown that visual modelling of chromatic perceptual differences (i.e., JNDs) using the receptor-noise limited model (Vorobyev and Osorio 1998) can reliably predict the likelihood of a host recognizing and rejecting a foreign egg (Cassey et al. 2008; Avilés et al. 2010; Honza and Cherry 2017). Therefore, we chose a “blended” blue/yellow (blue R,G,B = 45, 169, 239; yellow R,G,B = 246, 236, 112) color combination with the lowest median chromatic JND value and range from natural robin eggs (median = 2.51 JND and range = 1.26 to 4.65 JND, Table 3.2). Photoreceptor relative quantum catches are listed in Table 3.1 and both achromatic and chromatic JNDs of model egg colors are listed in Table 3.2. Because JND values close to 1 are expected to be more difficult to discriminate between than JND values much greater than 1 (Vorobyev and Osorio 1998), we predicted robins would perceive our blended blue-yellow model egg as quite similar to natural robin eggs, but not as entirely indistinguishable from them (i.e., imperfectly mimetic). All avian visual modeling was done using the *pavo* v 2.4 package (Maia et al. 2019) in R v 4.0.2 (R Core Team 2020).

The ability to spatially resolve visual stimuli varies across the avian retina (Fernández-Juricic 2012). Many bird species, like the American robin, tend to have a center of acuity vision (i.e., fovea) with high spatial resolution ability, surrounded by peripheral retinal area with much lower spatial resolution ability (Moore et al. 2017). In the American robin, the fovea projects into the lateral visual field, whereas portions of

the retinal periphery project into the binocular field (PB and EF-J, unpublished data). A study of seven passerine bird species found visual acuity estimates from anatomical measurements of eye posterior nodal distance (PND) and photoreceptor diameter corresponded well with visual acuity estimates derived from behavioral experiments (Donner 1951). However, anatomically-derived estimates of visual acuity using only measurements of eye PND and photoreceptor diameter overestimate visual acuity because they do not account for spatial summation of visual input by retinal ganglion cells (Caves et al. 2018). A more accurate anatomically-derived estimate of visual acuity can be acquired by assuming that retinal ganglion cell density sets the upper limit on spatial sampling of visual information (Collin and Pettigrew 1989; Ullmann et al. 2012). Additionally, a recent study on hummingbirds found a match between behavioral and anatomically-derived (based on retinal ganglion cells) estimates of visual acuity (Goller et al. 2019). Therefore, we used an anatomically-derived estimate of American robin visual acuity using measurements of eye PND and retinal ganglion cell densities (see Fernández-Juricic et al. 2019 for acuity calculation) to design the stimuli used in our experiments. Counts of retinal ganglion cells were indiscriminate of cells' responses to chromaticity, and therefore our acuity estimates are generalized and do not account for wavelength-specific (i.e., chromatic) visual acuity, which is often weaker than achromatic visual acuity in birds (Lind and Kelber 2011; Potier et al. 2018).

Peak visual acuity of the robins is estimated to be 14.54 cycles/degree for lateral (foveal) vision and 8.75 cycles/degree for binocular vision (peripheral to the fovea) (using retinal ganglion cell counts and eye axial diameter; PB and EF-J, unpublished data). We used the minimum angle of resolution calculated from robin peak foveal visual acuity (minimum angle of resolution = $0.069^\circ = \frac{1}{14.54\text{CPD}}$) to generate a range of 11 checkered square patterned eggs with visual angles spanning both above and below the minimum angle of resolution at various probable viewing distances (1 to 30cm, Fig. 3.2A). All visual angles were calculated using Eqn 1.0, where object size is the inter-square distances of the checkered patterns and object distance ranged between 1-30 cm. Viewing distances were roughly estimated as the distance from a robin's eye, while the robin views an egg using both lateral and binocular vision, to the surface of the model egg using video data of robins viewing eggs from Hauber et al. (2019) (e.g., see <https://youtu.be/GBTML1zcqQA>) (Fig. 3.2A). Checkered pattern designs were all 6 cm x 6 cm in total area and inter-square distances were 0.150 mm, 0.199 mm, 0.299 mm, 0.397 mm, 0.594 mm, 0.845 mm, 0.982 mm, 1.177 mm, 1.463 mm, 1.936 mm, and 2.40 mm. We also created a "control" 0.0 mm pattern using a checkered pattern with 0.150 mm inter-square distances and the Gaussian Blur filter in ImageJ (Schneider et al. 2012) to blend the blue and yellow squares together and create a uniform blue-green colored stimulus (intended to mimic natural robin egg color).

Artificial egg design

We used 3D-printed, brown-headed cowbird-sized model eggs (2.25cm length x 1.69 cm width in size, “Cowbird egg smooth,” purchased from Shapeways, Inc., following Igic et al. 2015) for all experimental model eggs. Cowbird-sized eggs have been used in previous experiments and can be successfully grasped and removed from nests by adult robins (Luro and Hauber 2017). Experimental egg pattern designs were printed as 6 cm x 6 cm squares onto transferrable decal paper (see *Experimental Egg Pattern Design* above). We cut out, wrapped, and glued the decal patterns onto the model eggs in a vertical orientation towards the egg poles and used small curved scissors to trim away excess decal. Air bubbles were removed by gently pressing the decals by hand until the pattern was flush and smooth on the surface of the model egg. Eggs were left to dry for at least 48 hours before being placed into wild robins’ nests in the field.

Egg rejection experiments

We searched for active robin nests in Champaign County, Illinois, USA, during May through June of 2019. Upon finding a nest with at least two eggs, we recorded the current clutch size, added a randomly chosen model egg into the nest, and monitored the nest daily until the artificial egg went missing from the nest (rejected) or up to 3 days from egg insertion if the egg remained in the nest (accepted; sensu Luro and

Hauber 2017). Adult robins were not captured or marked for this study but we visited three distant sites (more than 5 km apart), used multiple simultaneously active nests within each site, and tested each nest once with a single model egg treatment to reduce biological non-independence. Nest abandonment is not a response to experimental parasitism in robins (Croston and Hauber 2014a), and so data from abandoned ($n = 4$) or depredated nests ($n = 2$; out of 33 total nests tested) were not included in the analyses. Sample sizes for each treatment were as follows: control pattern, $N = 2$; 0.150 mm, $N = 2$; 0.199 mm, $N = 1$; 0.299 mm, $N = 2$; 0.397 mm, $N = 3$; 0.594 mm, $N = 2$; 0.845 mm, $N = 1$; 0.984 mm, $N = 2$; 1.177 mm, $N = 3$; 1.463 mm, $N = 2$; 1.936 mm, $N = 2$; and 2.40 mm, $N = 5$.

Statistical modeling

We used the *brms* v. 2.13 package (Bürkner 2017) in *R* v 4.0.2 to run a nonlinear logistic regression model as an approximate psychometric model (Kirwan and Nilsson 2019) predicting robins' rejection/acceptance responses to checkered model eggs placed into their nests. Our model included the inter-square distance (mm) of the model egg as the only predictor of robins' egg rejection responses, and rejection response probabilities were bounded between 0.15 and 0.85. We set a lower bound at 0.15 because we have previously shown that American robins reject cowbird-sized robin-mimetic blue-green colored eggs at a non-zero rate at a minimum rate around 15%

(Luro et al. 2018) and we set an upper bound at 0.85 because Croston and Hauber (2014b) found both blue and yellow colored cowbird-sized eggs were rejected at rates no greater than 85. We included priors (Gelman et al. 2017) for the model intercept (Normal: $sd = 1.5$, $location = 0$) and effect of pattern inter-square distance on rejection responses (Student T: $df = 10$, $location = 1$, $scale = 1.5$). The model intercept prior was chosen to reflect the range of probable values on the logit scale, while also reducing bias of the prior predictive density toward probabilities of 0 and 1 (McElreath 2020). Our prior for model egg pattern inter-square distance is informed by previous work showing that robins are more likely to reject eggs with more apparent patterning and contrast (Dainson et al. 2017), under the assumption that experimental eggs with greater inter-square distances should be more readily discriminated from natural robin eggs (Figs. 3.1, 3.2).

Finally, we determined the approximate inter-square distance rejection threshold (inter-square distance at which rejection probability is 0.5) by simulating posterior fitted values from the most accurate model for experimental egg inter-square distances between 0-2.4mm at 0.001mm increments and calculating the posterior median and 75% credible interval of inter-square distance for posterior fits with rejection probability = 0.5. We used 50% as our arbitrary rejection threshold because the median clutch size for robin nests in our experiments was 3—therefore if robins were only responding to the

addition of an egg into their nests and randomly guessing which egg to reject, they would correctly reject our model egg approximately 25% of the time. Thus, using a 0.5 probability of egg rejection as a threshold omits the possibility of random guessing while also selecting for a model egg pattern that is not readily discriminated and rejected (i.e., > 0.5 probability of rejection).

Results

We used data on the outcomes of the egg rejection experiments at $N = 27$ different nests with a median of $N = 2$ experimental replicates per model egg type for all 11 different checkered model egg treatments and the control model egg (0.0 mm inter-square distance). We obtained ≥ 10000 effective samples for each model parameter and all models' Markov Chains (MCMC) successfully converged ($R_{\text{hat}} = 1$ for all models' parameters).

Robin egg rejection responses increased with larger model egg blue-yellow inter-square distances (mm) (posterior median $\pm$ median absolute deviation [95% highest-density interval] = 0.92 ± 0.56 [-0.18, 2.05]) (Fig. 3.3). However, there was a fair amount of uncertainty in the model's predictive reliability (posterior median Bayes $R^2 \pm$ median absolute deviation [95% credible interval] = 0.08 ± 0.08 [0.01, 0.22]; where Bayes R^2 is the model predicted variance divided by the sum of model predicted variance and model error variance, see Gelman et al. 2019). Finally, we found that the approximate 50% egg

rejection threshold for robins' responses to the blue-yellow checkered model eggs was 1.18mm, corresponding to a visual angle range of 0.118° to 0.0118° from viewing distances of 1 cm and 10 cm respectively, with this approximation having a wide range of credible values (approximate 50% threshold posterior median [75% credible interval] = 1.18 mm [0.56 mm, 1.76 mm]). Given a 50% egg rejection threshold pattern inter-square distance of 1.18mm, the pattern should begin to blend from the robin visual perspective using either lateral (foveal) or binocular (peripheral) vision at viewing distances $> 3\text{cm}$.

Discussion

Our results suggest that American robins respond predictably to spatial chromatic contrasts when rejecting a foreign egg from the nest. Egg rejection responses were best predicted by inter-square distances of the blue-yellow checkered patterns of model eggs alone (Table 3.1), as robins were more likely to reject model eggs with larger inter-square distances (Fig. 3.3). These findings are consistent with the prediction that the combination of blue and yellow colored squares of our model eggs likely appears similar to natural blue-green robin eggs from the robin's perspective when robins are unable to completely resolve the colored squares as separate features (i.e., when the robin visual angle is below the minimum resolvable angle, Fig. 3.2).

Most passerine birds, including American robins, have laterally placed eyes and often move both their eyes and head when viewing an object of interest to align their

high-acuity foveal vision, or align their eyes to gaze at an object using binocular vision with lower acuity perifoveal retinal regions of the eyes (Land 2014; Moore et al. 2017). The distance at which robins view eggs in the nest, along with their visual acuity when viewing eggs with lateral (foveal) or binocular (peripheral) vision, is equally important for putting our results into context. For instance, if robins consistently inspect their eggs using either lateral or binocular vision from distances > 3 cm, then it is very likely that robins only partially resolved the blue and yellow squares as separate features for all our experimental model eggs (Fig. 3.2, both foveal and peripheral minimum resolvable angle lines for the American robin).

Previous work has shown that, generally, birds have much better spatial resolution for achromatic rather than chromatic patterns (Osorio et al. 1999; Lind and Kelber 2011; Potier et al. 2018). Secondly, birds may discriminate between visual stimuli using chromatic and achromatic contrasts separately in response to stimulus features and lighting conditions (Osorio et al. 1999; Jones and Osorio 2004). Osorio et al. (1999) found that chicks (*Gallus gallus*) discriminate between smaller patterns and textures using achromatic contrasts, and discriminate larger patterns using chromatic contrasts. Our avian receptor-noise limited models suggest the chromatic contrasts of our experimental egg patterns were likely more salient than achromatic contrasts in American robin assessment and rejection of experimental eggs (Table 3.2, blended blue-

yellow and blue vs. yellow square). However, our experiments could not directly disentangle the relative contribution of achromatic versus chromatic visual channels. No study to date has exclusively tested the contribution of egg achromatic contrast alone to brood parasite egg recognition using experimental eggs. Therefore, additional experiments testing brood parasite host responses to achromatic egg features, using a range of white-grey-black eggs (similar to Hanley et al. 2017's methods) and a range of black and white checkered eggs (similar to our methods here) are warranted.

Other factors expected to affect the ability of robins to resolve spatial chromatic contrasts of our model eggs include: pupil size, photoreceptor arrangement and spacing, and potential variation in the spectral profile of light illuminating the eggs and nest (Land et al. 2012; Cronin et al. 2014). Characterizing these visual properties in American robins would make it possible to more precisely test and model robin visual discrimination of egg features.

However, visual physiology alone, i.e., modeling visual perception using ocular anatomy and retinal physiology, cannot wholly predict egg recognition and egg rejection behavior of avian brood parasite hosts (Stoddard and Stevens 2011; Croston and Hauber 2014b; Hanley et al. 2017; Manna et al. 2017). Upon gathering visual information, brood parasite hosts likely use a combination of cognitive decision rules when recognizing and deciding to reject a foreign egg, including counting eggs in the

nest (Lyon 2003), rejecting the most dissimilar egg amongst all eggs in the nest (Moskát et al. 2010), and comparing a foreign egg appearance against an internal representation of own egg appearance (Stevens et al. 2013).

Our study also demonstrates the utility of avian egg recognition experiments using model eggs for testing mechanisms fundamental to visually-guided behaviors. Critically, the egg rejection behavior analyzed here is a fitness-relevant behavior for American robins because a small minority of robins do accept brown-headed cowbirds eggs laid in their nests (Lowther 1981), and raising parasitic cowbird nestlings reduces the fledging success of robin nestlings (Croston and Hauber 2015a). Surprisingly, many host species that are frequently parasitized by avian brood parasites do not reject foreign eggs laid into their nests even when there is a significant fitness cost to raising unrelated brood parasitic nestlings (Medina and Langmore 2016).

Our results demonstrate that host visual acuity and egg viewing behavior has the potential to limit the ability to detect and discriminate between spatial and chromatic features of eggs. Specifically, hosts can gather more detailed and accurate visual information about egg coloration and patterning by viewing eggs in their nest more often with their high acuity foveal vision or viewing eggs from shorter distances to increase the visual angles of the eggs' patterning. For example, common cuckoos (*Cuculus canorus*) have evolved better egg color and pattern mimicry for host species

that have robust egg rejection defenses (Stoddard and Stevens 2010, 2011). Perhaps cuckoo hosts that are skillful egg rejecters have also evolved egg viewing behaviors to better resolve the chromatic and spatial patterning of eggs in their nest, whereas species exhibiting weaker egg rejection responses to foreign eggs have not. Importantly, future experimental studies directly linking predictions derived from visual modeling with behaviors that have known fitness consequences will greatly advance the field of visual ecology (Luro and Hauber 2020).

Overall, our combined modeling and experimentation revealed that American robin acuity can predict egg rejection responses to foreign eggs, and spatial chromatic contrasts of eggs may be an important visual cue used by birds when viewing and recognizing eggs in their nests. The implication is that visual acuity can impose limits on egg recognition ability in hosts, an important task with considerable evolutionary fitness consequences for hosts of avian brood parasites. Specifically, resolving power constraints of the hosts could be exploited by brood parasites to minimize host detection of parasitic eggs. Finally, failing to recognize differences in visual perception between ourselves and non-human animals is notoriously common and problematic in comparative visual perception studies (Caves et al. 2019). Comprehending differences in visual acuities between ourselves and other species may be especially difficult, considering humans have relatively high visual acuity (Caves et al. 2018). Thus, our

results provide a compelling example of how our own biases in the detection, perception and processing of visual information may be vastly different from those of birds.

Figures and Tables

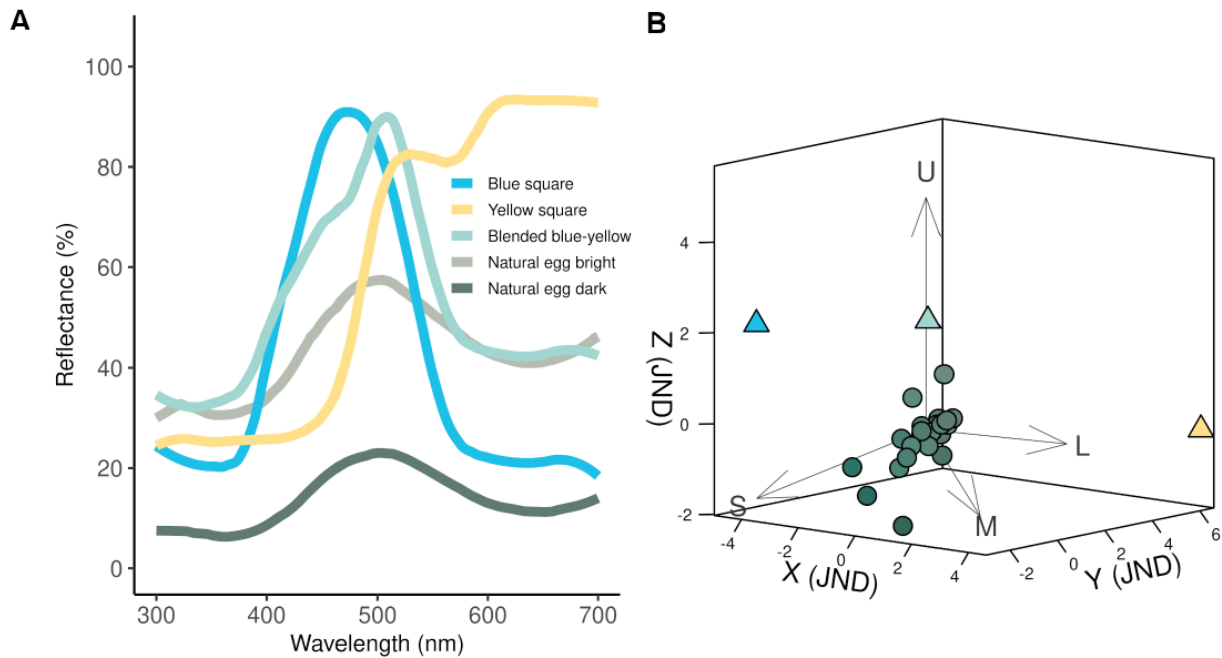


Figure 3.1 Avian visual modeling of experimental egg colors. **A** Representative reflectance spectra of blue square, yellow square, blended blue-yellow checkered pattern and two natural robin eggs. **B** 3D Euclidian distance Just Noticeable Difference (JND) plot of blue square (triangle), yellow square (triangle), blended blue-yellow checker pattern (triangle) and N=22 natural robin eggs (circles). Relative distance of points to each other are in units of JNDs, arrows represent each avian cone photoreceptor type: *U* = ultraviolet-sensitive cone, *S* = shortwave-sensitive cone, *M* = mediumwave-sensitive cone, *L* = longwave-sensitive cone. Natural robin egg reflectance spectra on the **A** were used from English and Montgomerie (2011) for “bright” and “dark” representative robin egg reflectance spectra, and robin egg reflectance spectra from Croston and Hauber (2015b) were used for the calculation of JND values.

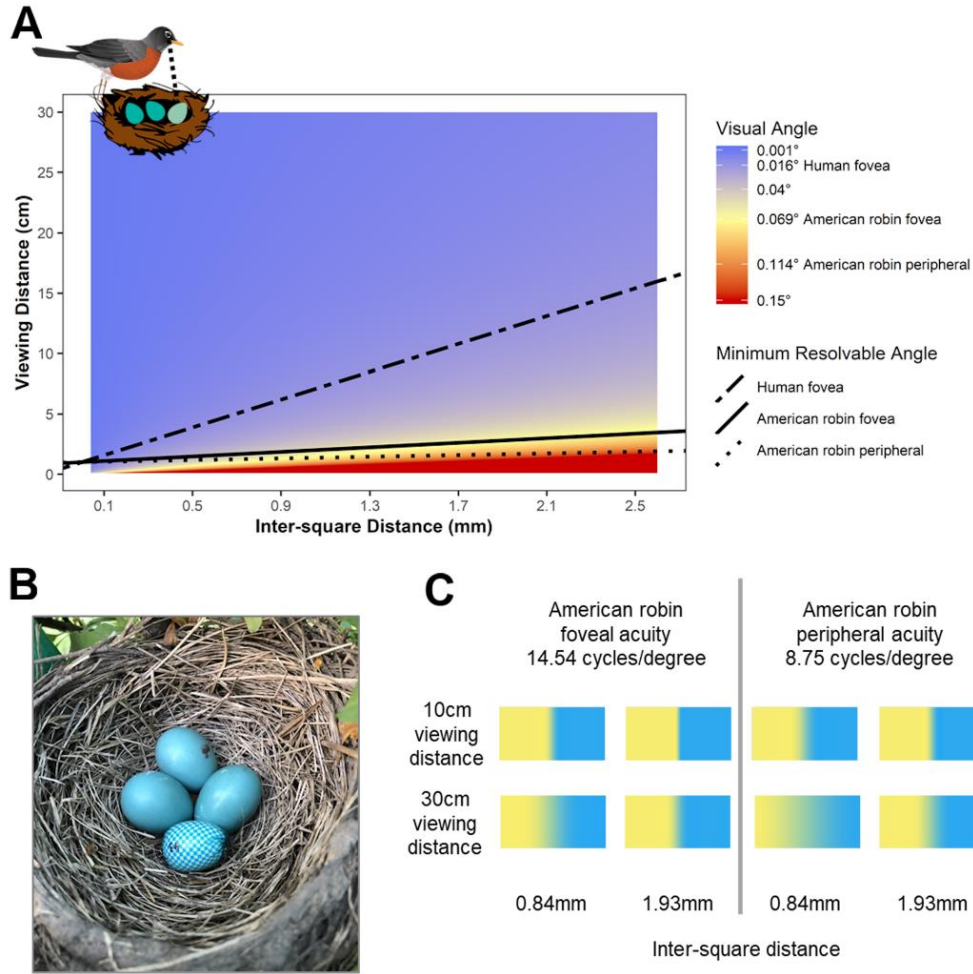


Figure 3.2 **A** Visual angles of model eggs from the human perspective (dot-dashed line, 60 cycles/degree acuity), and from the American robin (*Turdus migratorius*) perspective using either lateral (solid line, foveal acuity, 14.54 cycles/degree) or binocular (dotted line, peripheral acuity, 8.75 cycles/degree) vision. Visual angles were calculated from modelled pattern inter-square distances and viewing distances using *Eqn. 1.0*. We predicted model egg color should appear similar to natural robin egg color when the robin's visual angle is below the foveal and peripheral minimum resolvable angles as visual angles lower than the minimum resolvable angle should cause image blending of blue and yellow squares from the robin's perspective. **B** Photo of an experimental egg (egg towards bottom of nest) placed into a robin nest. **C** Acuity-corrected simulated images of experimental egg patterns as viewed by an American robin from 10 and 30cm distances using either foveal or peripheral vision. Acuity-corrected images were modeled using *AcuityView* image transformations (Caves and Johnsen 2017) in *ImageJ* (van den Berg et al. 2020).

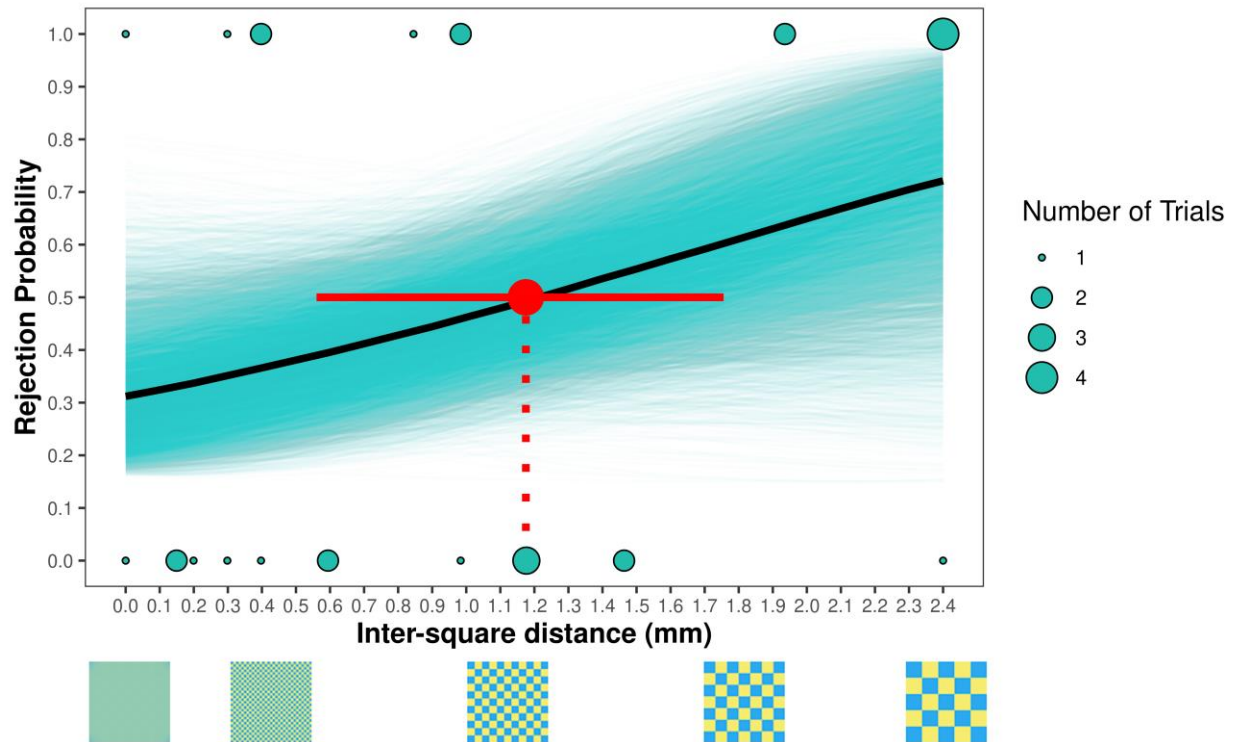


Figure 3.3 Model-fitted probabilities of American robin egg rejection responses to blue-yellow checkered model eggs. Blue-green lines are simulated logistic regression model fits for $N = 10,000$ posterior draws; black line represents the median of all posterior draws. Model egg inter-square distance between blue and yellow squares at which robins are predicted to reject model eggs at a 0.5 probability is indicated by red 75% credible interval and posterior median (posterior median [75% credible interval] = 1.18 mm [0.56 mm, 1.76 mm]). Inter-square distances of several representative checkered pattern images are true to the scale of the x-axis.

Stimulus	Relative Quantum Catch									
	UVS		SWS		MWS		LWS		Double-Cone	
<i>Natural robin egg (median from N = 22 eggs)</i>		0.250		0.370		0.260		0.130		0.180
<i>Blended blue- yellow</i>		0.350		0.330		0.200		0.120		0.570
<i>Blue square</i>		0.330		0.440		0.160		0.070		0.420
<i>Yellow square</i>		0.230		0.220		0.290		0.270		0.750

Table 3.1 Photoreceptor relative quantum catch values for experimental pattern colors and natural robin eggs (median of N=22 eggs). Quantum catch values of ultraviolet-sensitive (UVS), short wave sensitive (SWS), medium wave sensitive (MWS) and long wave-sensitive (LWS) cones sum to 1. Lollipop charts are of log2-transformed values of the stimulus quantum catch value: natural robin egg quantum catch value ratio.

Comparison	Stimulus	Chromatic JND median (range)	Achromatic JND median (range)
JND from natural robin eggs	<i>Blended blue-yellow</i>	2.5 (1.3, 4.6)	11.7 (7.9, 17)
	<i>Blue square</i>	5.3 (4.2, 5.9)	8.7 (4.8, 13.9)
	<i>Yellow square</i>	7.8 (7, 10.2)	14.5 (10.7, 19.8)
JND within pattern	<i>Blue vs. yellow square</i>	12.5	5.9

Table 3.2 Chromatic and achromatic contrasts (as “just-noticeable differences,” JND) of experimental egg pattern colors compared to natural robin eggs (N=22 eggs from Croston and Hauber 2015) and to each other. JND values were calculated using a receptor-noise limited visual model of the European blackbird (*Turdus merula*) visual system (Vorobyev and Osorio 1998; Hart et al. 2000), a congener of the American robin (*Turdus migratorius*).

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CHAPTER 4: AVIAN DIET AND FORAGING ECOLOGY CONSTRAIN FOREIGN EGG RECOGNITION AND REJECTION ⁴

Abstract

Egg rejection is a common and effective defense against avian brood parasitism in which the host either marginalizes or removes the parasitic egg or deserts the parasitized clutch. The ability to recognize and reject a parasitic egg depends on bill morphology, sensory systems, and cognition, all of which are also shaped by other selective processes, such as foraging. This begs the question whether specific phenotypes associated with different foraging strategies and diets may constrain or facilitate egg recognition and rejection. Here, we propose a novel hypothesis that host species phenotypes related to foraging ecology and diet may impose morphological and visual sensory constraints on the evolution of egg recognition and rejection. We conducted a comparative analysis of the adult diets and egg rejection rates of 165 current host and non-host species. We found that species have significantly higher egg rejection rates when they (i) consume an omnivorous or animal and fruit dominated diet rather than seeds and grains, (ii) forage arboreally rather than aerially or on the ground, or (iii) possess relatively larger body sizes. Though correlational in nature these

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results suggest phenotypes related to specific diets and foraging ecologies may differentially constrain or facilitate evolution of host egg rejection defenses against avian brood parasitism.

Introduction

Obligate avian brood parasitism is a breeding strategy wherein a parasitic species lays its eggs into the nests of other species that provide parental care for the unrelated offspring (Davies 2000). Brood parasitism, by definition, is detrimental to the host's survival and reproductive effort, because parasitic chicks may require longer and more intensive provisioning than host broods, and host eggs or nestlings may be outcompeted, or otherwise eliminated, by the hatchling parasite (Soler 2017).

To reduce or forego many of these costs of avian brood parasitism, egg rejection is a common antiparasitic defense in which a host either removes the parasitic egg from the nest (Rothstein 1975) or deserts the parasitized nest (Hosoi and Rothstein 2000). The likelihood of the hosts evolving an egg rejection defense is predicted to be greater when the cost of raising brood parasite nestlings is higher (Medina and Langmore 2015). Yet, many host species do not reject foreign eggs, despite not only facing high costs of parasitism, but also having their own eggs predicted to be readily perceived as visually different from brood parasite eggs (Stoddard and Stevens 2011), and a demonstrated physical capability of removing objects from the nest as large and heavy as foreign eggs

(Rothstein 1975). There are several adaptive hypotheses for why some host species have not evolved egg rejection, including the evolutionary equilibrium hypothesis, which suggests that the cost of breaking or mistakenly rejecting own eggs counterweighs the benefits of rejecting brood parasite eggs (Lotem et al. 1995). A non-adaptive alternative is the evolutionary lag hypothesis which proposes that not enough time has passed since the onset of a coevolutionary host-brood parasite arms race for the host to evolve egg rejection defenses (Rothstein 1990).

A novel, non-exclusive hypothesis in concordance with evolutionary lag for the absence of host egg rejection is that different host species phenotypes may differentially constrain (Schwenk 1995) or facilitate the evolution of egg rejection over a period of time due to perceptual and physical limitations, despite the costs of raising unrelated offspring. Specifically, foreign egg recognition is only possible if the host is capable of perceiving brood parasite eggs as different from its own eggs, typically using visual cues (Stoddard and Hauber 2017). Furthermore, small host bill size or lower body mass may also limit the ability to remove a brood parasite egg from the nest (Rohwer and Spaw 1988).

How might species phenotypes have been shaped in a manner that either constrains or facilitates the evolution of egg rejection? Foraging ecology and diet play predominant roles in the evolution of avian body size and cranial and bill morphology

(Kilner 2006; Kulemeyer et al. 2009; Pigot et al. 2016; Felice et al. 2019; Pigot et al. 2019), visual perception and other sensory systems (Garamszegi et al. 2002; Martin 2017), and cognition decision rules (Mendelson et al. 2016). All of these morphological and neural traits may directly shape a species ability to recognize and/or remove a foreign egg from the nest (Rohwer and Spaw 1988; Stoddard and Hauber 2017). For example, granivorous bird species generally have bills that are wide in depth and short in length (Pigot et al. 2019); these may be unsuitable for piercing or grasping and removing foreign eggs (Rohwer and Spaw 1988). Similarly, granivorous birds may have visual acuities that are poorer at the same distances than those of the visually guided insectivores which feed on highly mobile and/or often cryptic prey (Dolan and Fernández-Juricic 2010; Moore et al. 2015). In turn, frugivorous birds may be more sensitive and attuned to color differences than are granivorous birds, because chromatic differences are reliable long-distance cues for identifying ripe fruits (Cazetta et al. 2009). For example, trichromatic color vision is associated with frugivorous diets across primate species (Osorio and Vorobyev 1996) and is also associated with greater ability to detect fruits at long distances as well as a wider breadth of discriminable fruit colors in trichromatic individuals of polymorphic tri/dichromatic capuchin populations (Melin et al. 2014). Alternatively, achromatic features including shape and pattern contrast are likely more reliable than chromatic cues in identifying cryptic seeds on the ground for

granivorous birds (Porter 2013). Therefore, we predict that insectivorous and frugivorous birds may possess visual sensory and perceptual traits better suited than those of granivorous birds for visually discriminating foreign eggs in their nests. The specific visual tasks and mechanisms leading to differences in foreign egg discrimination ability may include recognizing egg maculation pattern and color differences between own and foreign eggs (Dainson et al. 2017). The perception and processing of these cues, in turn, could be constrained by visual spatial resolution (i.e., acuity, see Caves et al. 2018) for a given species, and/or perceiving and attending to eggshell background color differences. Potential neurosensory mechanisms constraining color and spatio-chromatic vision in birds include species-specific patterns of photoreceptor opsin and oil droplet tuning, relative photoreceptor densities, photoreceptor mosaic variation across the retina, and neural color opponency channels (Hart 2001; Cassey et al. 2008; Spottiswoode and Stevens 2010; Stoddard et al. 2014). Here, we test how foraging ecology and diet-related phenotypes may influence evolutionary trajectories of host egg rejection defenses using phylogenetic comparative methods.

Methods

Data

We collected data from published avian egg rejection studies for as many species as we could find (N=174), using “egg rejection” as key words, then searched for published diet data for the respective species and generated a final dataset of both egg rejection and categorized diet data, matched with a set of 100 phylogenetic hypotheses from birdtree.org (Jetz et al. 2012) for N=165 species. For each species, our complete dataset included: weighted average egg rejection rate (weighted by study experiment sample sizes), average body mass (g) (from del Hoyo et al. 2017), average tarsus length, and bill length, width, and depth (mm) (from the literature, mainly Ricklefs 2017). Additional categories included diet and forage zone (from del Hoyo et al. 2017), the associated obligate avian brood parasite taxon (*Cuculidae* and *Molothrus*), and whether the species is a known current host of its associated brood parasite, i.e., “host status” (from Johnsgard 1997; Ortega 1998). Diet categories included: “frugivore” (N=3), “granivore” (N=13), “insectivore” (N=54), “insectivore/frugivore” (N=37), “insectivore/granivore” (N=27), “nectarivore/insectivore” (N=5), and “omnivore” (N=26) and were assigned using food and feeding descriptions from the *Handbook of the Birds of the World* (HBW) (del Hoyo et al. 2017). Omnivores were assigned as those species with food and feeding descriptions including the term “omnivorous.” For further analyses,

where diet category was included as a predictor, strict frugivores (N=3) and nectarivores/insectivores (N=4) were excluded due to low species numbers. We also included foraging zone as one of three categories: “ground,” “arboreal” or “aerial.” Categories followed the foraging classification system of the Cornell Lab of Ornithology online bird guide (<https://www.allaboutbirds.org/guide/>). Foraging behavior descriptions from HBW were also used: “*ground*” was indicated if a species’ description included “forages mainly on ground,” “*arboreal*” was indicated if the description included “forages in canopy/trees/shrubs,” and “*aerial*” was indicated if the description included “forages while in flight; forages x meters above ground.”

Discrete assigned diet categories may not adequately capture relevant degrees of variation among proportions of food types within avian diets. Therefore, we also collected published quantitative measurements of species’ diets for N=96 species. We collected percent diet composition for adults, mainly measured as percent stomach content volume (N=81), percent fecal content volume (N=8), or stomach or fecal content frequency proportion (N=7). We separated major food sources into three major categories: Animal, Fruit, and Seed. Finally, we followed Olson et al. (2008) and used a 7-point scoring system to bin Animal, Seed, and Fruit diet proportions for all species. Scoring was necessary to reduce the potential measurement error due to between-study differences in diet categorization, and to include verbal descriptions of species’ diets in

cases where precise numerical estimates were not provided. Scores for Animal, Fruit and Seed diet were assigned by the percent contributed to species' entire diets: 0-1% scored as 1, 1.1-16% scored as 2, 16.8-33% scored as 3, 33.4-50% scored as 4, 50.1-66.7% scored as 5, 66.8-83.4% scored as 6, 83.5-100% scored as 7. For each of these 96 species, our dataset again included: weighted average egg rejection rate (weighted by study sample sizes), average body mass (g), average wing chord (mm), average tarsus length (mm), average length of bill culmen (mm), average bill width and depth (mm) (from Ricklefs 2017), animal diet, seed diet, fruit diet, forage zone, associated brood parasite taxon, and whether the species is a known current host of a brood parasite.

We included both species considered to be evolutionarily current hosts or “non-hosts” (*sensu* Medina et al. 2017) in all analyses together because the focal interest of this study is to directly examine the relationship of the ability to recognize and reject foreign eggs from the nest with foraging ecology phenotypes, and not with the influence of host-brood parasite coevolution systems *per se*.

Comparative phylogenetic analyses

For the full dataset, we ran five separate Bayesian *MCMCglmm* models (Hadfield 2010) predicting egg rejection rates for N=165 species. We ran all *MCMCglmm* models over 100 phylogenies using the *mulTree* package (Guillerme and Healy 2018) in R (R Core Team 2017), using a weakly informative parameter expanded prior ($V=1$, $\nu=1$,

prior mean $\alpha.\mu=0$, $\alpha.V=10^3$) (Hadfield 2012; Kay 2019), setting the number of MCMC generations to 4,000,000, the thinning interval to 1500, and the burn in period to 100,000. Models were run in parallel over 7 chains to obtain ≥ 1500 samples per chain. Model convergence between model chains was assessed using the Gelman-Rubin statistic, the potential scale reduction factor (PSR), and all were models required to have a PSR below 1.1 (Healy et al. 2014).

We ran five separate models with the full dataset (N=165): 1) testing the influence of species foraging zone (*aerial*, *arboreal* or *ground*), 2) testing for differences among associated brood parasite taxon and host status (*Cuculidae* vs. *Molothrus*, current *host* vs. *non-host*, and their interaction), 3) testing the influence of species categorized diet phenotypes with *insectivores*, the most common diet phenotype, set as the comparison group (i.e., *insectivore/frugivore*, *insectivore/granivore*, *granivore*, and *omnivore* vs. *insectivore*), and two separate models testing the influence of categorized diet phenotypes in 4) current *Molothrus* hosts only (N=56), and 5) current *Cuculidae* hosts only (N=53). Log₁₀-transformed body mass was also included as a predictor in all models, except for the host status model, to account for the influence of species body sizes. For the current hosts only diet category models, strict *granivores* were excluded due to low species numbers in the separate samples of *Molothrus* (N=3 *granivores*) and *Cuculidae* hosts (no *granivores*).

For the reduced dataset of N=96 species, we first performed two separate phylogenetic principal component analyses (pPCA) (Revell 2009), 1) morphology-based pPCA on $\log_{10}$ -transformed measurements of body mass, bill length, wing chord, tarsus length, bill length, bill width and bill depth. The first three components explained 87% of all variation in species bill and body morphologies, and we named these components based on interpreted axes of their loadings: PC1-Size, PC2-Tarsus vs. Bill Width, and PC3-Bill Length (Table 4.1). Then, we ran a separate diet-based pPCA using Animal, Seed and Fruit diet scores. We included all three components from this pPCA and named them: PC1- Animal vs. Seed diet, PC2-Fruit vs. Seed diet (multiplied by -1 to interpret positive values as higher proportion of fruit diet), and PC3-Omnivory (Table 4.2). Phylogenetic signal was high for both the morphology (Pagel's $\lambda=0.87$) and diet pPCA (Pagel's $\lambda=0.85$), indicating that closely related species share highly similar morphologies and diets. We ran a single *MCMCglimm* model predicting species egg rejection rates with predictors of PC1-Size, PC2-Tarsus vs. Bill Width, PC3-Bill Length, PC1-Animal vs. Seed, PC2-Fruit vs. Seed and PC3-Omnivory over 100 phylogenies using the same model prior and parameters as described above. Egg rejection rates were mean-centered and scaled to one standard deviation from the mean for all models.

Results

All *MCMCglmm* models passed convergence ($PSR < 1.1$) and produced ≥ 1500 posterior estimates per each predictor from 100 iterations of each model, where each iteration was run with a separate phylogenetic hypothesis. Bayesian inference provides an estimate of the probability of a hypothesis given the data (Ellison 2004). We present the posterior mode and 95% highest density interval (HDI) of each model predictor as a point estimate and distribution of effect size to infer both the magnitude and direction of a predictor's effect on avian egg rejection responses. Our degree of certainty in the true existence of an effect for a predictor is expressed as a posterior probability for a negative, null, or positive influence of a predictor on avian egg rejection (for an overview of Bayesian inference, see Ellison 2004). Model predictor posterior modes and 95% HDIs were calculated from the highest density region of combined posterior distributions across all 100 model iterations using the *tidybayes* package in R (Kay 2019). Posterior probabilities of effects of predictors (Ellison 2004) having negative (P^-), null (P_0 , probability of predictor having an effect within an interval around zero), and positive (P^+) effects on egg rejection rates were calculated using the *BayesCombo* package in R (Contrino et al. 2017).

Categorized diet predicting egg rejection

For the full dataset models including all species associated with both *Molothrus* and *Cuculidae* brood parasites (both current host and non-host species included), the omnivorous (posterior mode=0.45, 95%-HDI=[0,0.90], $P(-|P0|P+)=0.04|0.2|0.77$, Fig. 4.1A) and insectivore/frugivore species (posterior mode=0.44, 95%-HDI=[0.05,0.84], $P(-|P0|P+)=0.03|0.15|0.82$, Fig. 4.1A) have higher egg rejection rates in comparison with mainly insectivorous species, while granivorous species have relatively lower egg rejection rates (granivore posterior mode=-0.63, 95%-HDI=[-1.30,0.05], $P(-|P0|P+)=0.75|0.21|0.04$; granivore/insectivore posterior mode=-0.49, 95%-HDI=[-0.93,-0.06], $P(-|P0|P+)=0.84|0.13|0.02$, Fig. 4.1A). Higher body mass is associated with higher egg rejection rates ($\log_{10}$ -Body Mass posterior mode=0.12, 95%-HDI=[-0.08,0.32], $P(-|P0|P+)=0.1|0.35|0.55$, Fig. 4.1A). Residual variance posterior mode=0.35, 95%-HDI=[0.16,0.59] (Fig. 4.1A). Phylogenetic variance posterior mode=0.90, 95%-HDI=[0.20,1.95] (Fig. 4.1A).

Forage zone predicting egg rejection

In comparison to ground foraging species, arboreal foragers have marginally higher egg rejection rates (posterior mode=0.21, 95%-HDI=[-0.14,0.56], $P(-|P0|P+)=0.11|0.36|0.53$, Fig. 4.1B), whereas no discernable pattern was found in the comparison between aerial and ground foragers (posterior mode=0.09, 95%-HDI=[-

0.65,0.79], $P\text{-}|P0|P+= 0.25|0.42|0.33$, Fig. 4.1B). In concordance with the diet category models, higher body mass is associated with higher egg rejection rates ($\text{Log}_{10}\text{-Body Mass}$ posterior mode=0.18, 95%-HDI=[-0.01,0.39], $P\text{-}|P0|P+=0.05|0.23|0.72$, Fig. 4.1B). Residual variance posterior mode=0.27, 95%-HDI=[0.11,0.52] (Fig. 4.1B). Phylogenetic variance posterior mode=1.64, 95%-HDI=[0.71,2.74] (Fig. 4.1B).

Associated brood parasite taxon and host status predicting egg rejection

Overall, species associated with *Cuculidae* brood parasites have lower egg rejection rates than species associated with *Molothrus* brood parasites (posterior mode=-0.86, 95%-HDI=[-1.44,-0.28], $P\text{-}|P0|P+= 0.97|0.03|0$, Fig. 4.2A), and current host species have lower egg rejection rates than non-host species (posterior mode=-0.46, 95%-HDI=[-0.90,-0.01], $P\text{-}|P0|P+= 0.79|0.18|0.03$, Fig. 4.2A). Accordingly, because both associated brood parasite taxon and host/nonhost status combined predict a substantial amount of variation in species egg rejection rates (interaction between associated brood parasite taxon and host status posterior mode=1.18, 95%-HDI=[0.55,1.80], $P\text{-}|P0|P+= 0|0|1$, Fig. 4.2A), we ran separate diet category models predicting egg rejection rates of current hosts of *Cuculidae* brood parasites and current hosts of *Molothrus* brood parasites. Residual variance posterior mode=0.28, 95%-HDI=[0.12,0.48] (Fig. 4.2A). Phylogenetic variance posterior mode=1.42, 95%-HDI=[0.67,2.46] (Fig. 4.2A).

Categorized diet predicting egg rejection in Cuculidae or Molothrus hosts

In *Cuculidae* hosts (N=53 species), larger body mass is marginally associated with lower egg rejection rates (Log₁₀-Body Mass posterior mode=-0.19, 95%-HDI=[-0.53,0.02], $P-|P0|P+=0.52|0.37|0.12$, Fig. 4.2B). In comparison with mainly insectivorous hosts, omnivores have marginally higher egg rejection rates (posterior mode=0.32, 95%-HDI=[-0.57,1.20], $P-|P0|P+=0.17|0.41|0.42$, Fig. 4.2B) and insectivore/frugivores have credibly higher egg rejection rates (posterior mode=0.47, 95%-HDI=[-0.10,1.04], $P-|P0|P+=0.06|0.27|0.67$, Fig. 4.2B). Granivore/insectivore *Cuculidae* hosts have marginally lower egg rejection rates than insectivore hosts (posterior mode=-0.33, 95%-HDI= [-1.26,0.60], $P-|P0|P+=0.43|0.41|0.16$, Fig. 4.2B). Residual variance posterior mode=0.23, 95%-HDI= [0.08,0.58] (Fig. 4.2B). Phylogenetic variance posterior mode=1.21, 95%-HDI=0.36,2.55] (Fig. 4.2B).

In *Molothrus* hosts (N=56 species), larger body mass is associated with higher egg rejection rates (Log₁₀-Body Mass posterior mode=0.26, 95%-HDI=[-0.02,0.54], $P-|P0|P+=0.05|0.23|0.72$, Fig. 4.2C). In comparison with mainly insectivorous hosts, omnivores have marginally higher egg rejection rates (posterior mode=0.44, 95%-HDI=[-0.37,1.24], $P-|P0|P+=0.12|0.37|0.51$, Fig. 4.2C) but insectivore/frugivore diet type does not credibly predict egg rejection rates (posterior mode=0.15, 95%-HDI=[-0.51,0.82], $P-|P0|P+=0.22|0.42|0.36$, Fig. 4.2C). Granivore/insectivore *Molothrus* hosts have

marginally lower egg rejection rates than insectivore hosts (posterior mode=-0.33, 95%-HDI=[-0.95,0.27], $P(-|P0|P+)=0.50|0.38|0.12$, Fig. 4.2C). Residual variance posterior mode=0.42, 95%-HDI= [0.14,0.78] (Fig. 4.2C). Phylogenetic variance posterior mode=0.46, 95%-HDI=[-0.02,2.54] (Fig. 4.2C).

Quantitative diet and egg rejection

In a subset of N=96 species for which we collected quantitative measures of diet, we found PC3-Omnivory to be negatively associated with egg rejection rates (posterior mode=-0.39, 95%-HDI=[-0.87,0.10], $P(-|P0|P+)=0.65|0.28|0.07$, Fig. 4.3), greater frugivory (PC2-Fruit vs. Seed*-1 posterior mode=0.20, 95%-HDI=[-0.31,-0.10], $P(-|P0|P+)=0|0|1$, Fig. 4.3) and consumption of animals (PC1-Animal vs. Seed posterior mode=0.08, 95%-HDI=[0,0.16], $P(-|P0|P+)=0.03|0.18|0.78$, Fig. 4.3) to be positively associated with higher egg rejection rates. For morphological predictors, larger species have higher egg rejection rates (PC1-Size*-1 posterior mode=0.02, 95%-HDI=[0.03,0], $P(-|P0|P+)=0.01|0.06|0.93$, Fig. 4.1) but PC2-Tarsus vs. Bill Width was not predictive of species egg rejection rates (posterior mode=0.01, 95%-HDI=[-0.02,0.05], $P(-|P0|P+)=0.19|0.42|0.39$, Fig. 4.3). Bill length, as captured by our pPCA, seems to have no direct association with egg rejection rates (PC3-Bill Length posterior mode=0, 95%-HDI=[-0.05,0.05], $P(-|P0|P+)=0.3|0.42|0.29$, Fig. 4.3). Residual variance posterior

mode=0.38, 95%-HDI=[0.18,0.66] (Fig. 4.3). Phylogenetic variance posterior mode=0.64, 95%-HDI= [0.01,1.73] (Fig. 4.3).

Discussion

Across all bird species sampled, omnivorous and frugivorous diet rather than insectivorous diet, insectivorous diet rather than granivorous diet, arboreal rather than aerial or ground foraging, and relatively larger body mass were predictive of more frequent rejection of foreign eggs (Fig. 4.1A, 4.1B). When accounting for the influence of host-brood parasite coevolutionary relationships, directional effects of all diet types on egg rejection rates were similar across hosts of both *Cuculidae* and *Molothrus* hosts (Fig. 4.2B, 4.2C), albeit to a less pronounced degree than when testing effects of diets on egg rejection in a combined dataset of both current hosts and non-hosts associated with both brood parasite taxa (Fig. 4.1A). Furthermore, these patterns between diet types and egg rejection rates remain consistent when diets are measured as quantitative diet proportions of diet categories, rather than discrete categorical traits (Fig. 4.3). The exception to consistency between qualitative and quantitative models is our “omnivorous” diet principal component score (i.e., PC3-Omnivory, which explains 2% of the variation in diet in the reduced N=96 dataset), as omnivorous diet was associated with higher egg rejection rates in the full qualitative models and lower egg rejection rates in the reduced quantitative model. However, this principal component likely did

not adequately characterize omnivorous diet and overall variation in diet because it was limited to broad categories of animal, fruit, and seed consumption alone while disregarding finer-scale food types within these categories. Finally, as with all comparative studies, the patterns detected here are correlational in nature (Price 1997), and future studies should explore both assumptions and direct causal linkages between foraging ecology, visual system traits, and egg rejection cues in avian obligate brood parasite host species.

An alternative explanation for the patterns we found between diet types and egg rejection rates may be that species with diets considered to be unsuitable for brood parasite nestlings are far less likely to be parasitized, and therefore do not need to evolve egg rejection defenses in the first place (Kozlovic et al. 1996; Turtumøygaard and Slagsvold 2010). Specifically, in our analyses diet unsuitability may explain the lack of egg rejection defenses we found in highly granivorous species (Fig. 4.1A, 4.2B, 4.2C). Indeed, a recent study found that common cuckoos *Cuculus canorus* prefer to parasitize host species that feed their nestlings insects over those that do not (Stokke et al. 2018), and *Molothrus* cowbird nestlings are known to depend on animal protein to survive and fledge from host nests (Mason 1986). However, contrary to this scenario, many potential host species consume mainly insects and feed highly insectivorous diets to their young during the breeding season and switch to eating mainly seeds and grains in the

nonbreeding season, and we account for these species in our analyses (i.e. “granivore/insectivore”). Additionally, adult and nestling diets can differ from one another during the breeding season. For example, adult starlings (*Sturnus vulgaris*) consume large amounts of fruit in the fall and winter, but their nestlings are not provisioned with fruits even when fruits are available during the nestling feeding period (Moeed 1980). Our data are drawn from descriptions and data available for adult birds only and, thus, they likely do not provide an accurate representation of diets fed to nestlings. Accordingly, our results are conservative in that they more directly apply to the foraging ecology hypothesis regarding adult phenotypes.

Our results suggest that, in addition to known effects of the degree of brood parasite egg mimicry (Stoddard and Stevens 2011) and the potential costs associated with being parasitized (Medina and Langmore 2015), phenotypic traits known to be associated with different foraging strategies and diets (e.g., visual sensory systems and morphology) may differentially constrain or facilitate the evolution of host species ability to recognize and reject a foreign egg by its color (Stoddard and Hauber 2017), maculation pattern, and/or shape and size. For example, visually-guided insectivorous birds possess visual fields predicted to be better suited for eye-beak coordination than birds that consume immobile food (Moore et al. 2015), and better eye-beak coordination may confer greater ability to physically remove a foreign egg from the nest.

Additionally, frugivory is facilitated by use of chromatic cues and object-background contrasts (Osorio and Vorobyev 1996; Cazetta et al. 2009) and the range of ripe fruits which can be consumed is limited by minimum bill gape-width (Wheelwright 1985), which may also carry over into discrimination between colors of own vs. foreign eggs as well as the ability to grasp and remove an egg from the nest (Rohwer and Spaw 1988). Body mass and eye size are highly and positively correlated in birds (Garamszegi et al. 2002; Burton 2008), and larger eye sizes are associated with higher visual acuities (Kiltie 2000; Cronin et al. 2014). Accordingly, the positive relationships we found between greater body mass and egg rejection rates may indicate that greater visual acuity, along with larger body and bill size, confers greater sensory and motor abilities to recognize and reject foreign eggs. For example, higher visual acuity may allow for greater ability to visually resolve egg patterning (e.g. egg spotting and scrawling) from a wider range of viewing distances. Importantly, although we found evidence that certain diet and foraging ecology-related phenotypes influence foreign egg recognition and rejection rates, our study does not directly examine specific sensory and physical mechanisms that may influence egg rejection defenses. Therefore, we suggest future comparative and experimental studies use our results as a guideline for examining suites of morphological, sensory or cognitive traits that may form a mechanism explaining the

patterns we found here (e.g., complete bill morphometrics, visual acuities, photoreceptor densities, color discrimination ability, etc.).

In summary, we provide exploratory support for the hypothesis that foraging ecology and diet may affect the evolutionary trajectory of egg rejection defenses in avian brood parasite hosts. Specifically, we predict current and future hosts of avian brood parasites that do not currently exhibit egg rejection defenses to more readily evolve egg rejection if they are omnivorous or frugivorous rather than insectivorous, insectivorous rather than granivorous, forage arboreally rather than in the air or on the ground and have a relatively large body size.

Figures and Tables

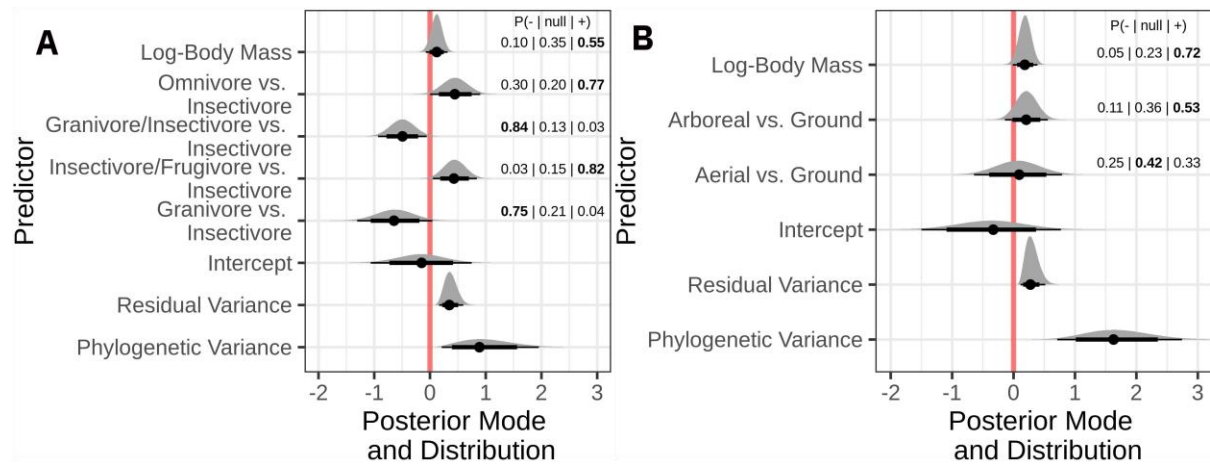


Figure 4.1 Full dataset models testing the influence of **A** diet and **B** forage zone on egg rejection rates in N=165 species. Posterior modes and distributions, their 95% highest-density intervals (thin line), 80% highest-density intervals (thick line), and posterior probabilities of having either negative, null or positive effects on egg rejection rates are presented. The highest posterior probability for the existence of an effect of a predictor having negative, null, or positive relationship with egg rejection rates are indicated in bold. No posterior probabilities are provided for the intercept, model residual variance, or phylogenetic variance because the intercept is centered towards zero and the variances are positive-only values.

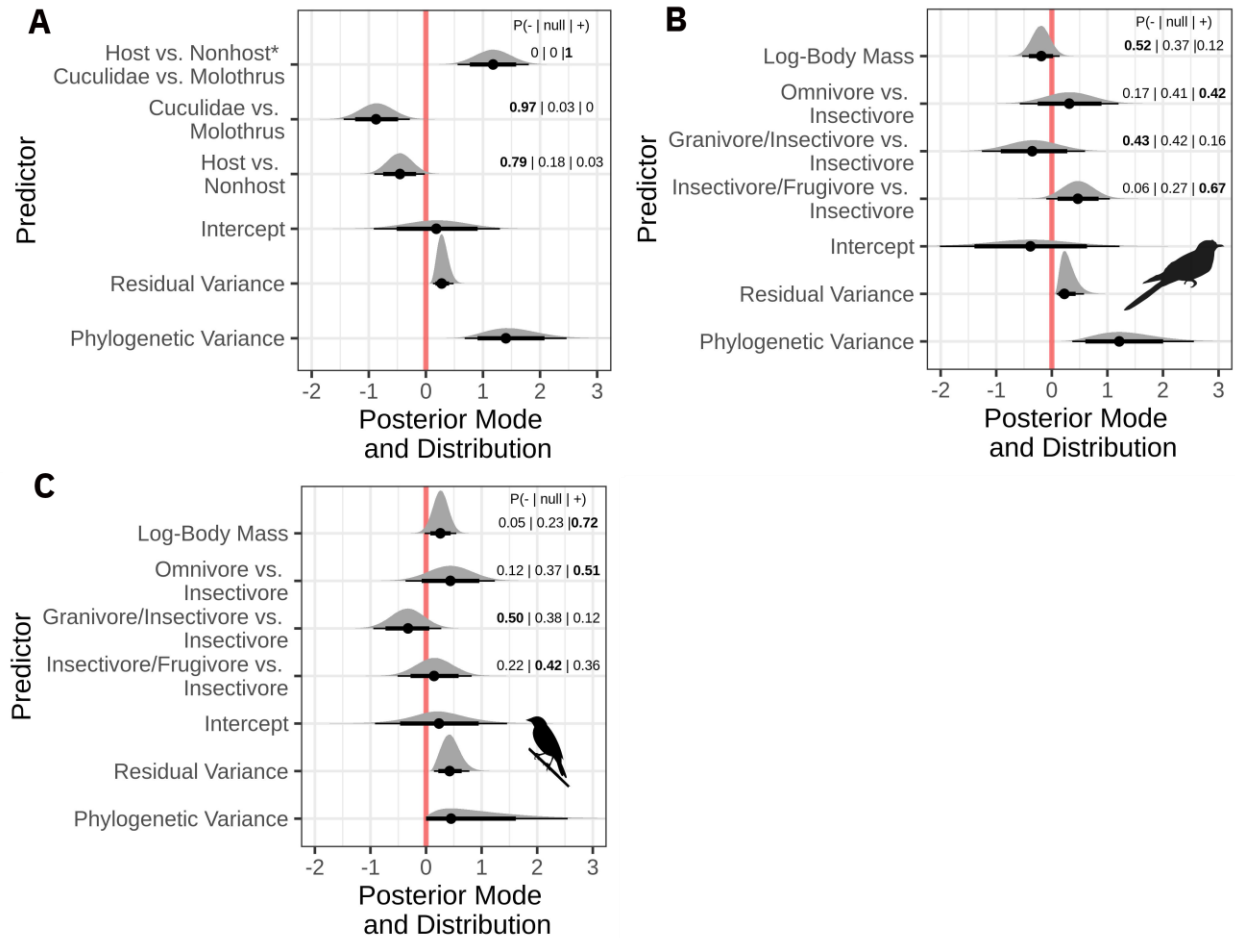


Figure 4.2 Full dataset model testing the influence of **A** associated brood parasite taxon and host status in N=165 species. Reduced dataset models testing the influence of diet on egg rejection rates in current hosts of **B** *Cuculidae* (N=53) and **C** *Molothrus* (N=56) brood parasites. Posterior modes and distributions, their 95% highest-density intervals (thin line), 80% highest-density intervals (thick line), and posterior probabilities of having either negative, null or positive effects on egg rejection rates are presented. The highest posterior probability for the existence of an effect of a predictor having negative, null, or positive relationship with egg rejection rates are indicated in bold. No posterior probabilities are provided for the intercept, model residual variance, or phylogenetic variance because the intercept is centered towards zero and the variances are positive-only values.

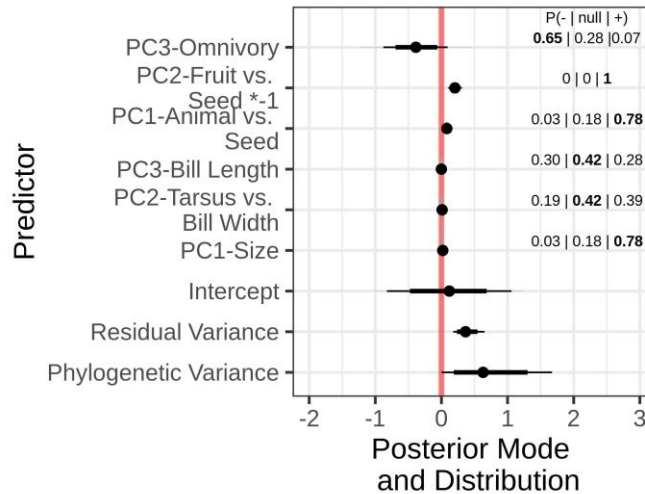


Figure 4.3 Reduced dataset model testing the effect of quantitatively measured diets on egg rejection rates in N=96 species. Diet data collected from published studies where physical contents of consumed items were observed (i.e., stomach or fecal content). Posterior modes and distributions, their 95% highest-density intervals (thin line), 80% highest-density intervals (thick line), and posterior probabilities of having either negative, null or positive effects on egg rejection rates are presented. The highest posterior probability for the existence of an effect of a predictor having negative, null, or positive relationship with egg rejection rates are indicated in bold. No posterior probabilities are provided for the intercept, model residual variance, or phylogenetic variance because the intercept is centered towards zero and the variances are positive-only values.

	PC1- Size	PC2-Tarsus vs. Bill Shape	PC3-Bill Length	PC4	PC5	PC6
Bill length	-0.76	0.24	0.56	-0.21	-0.03	0.04
Bill width	-0.74	-0.54	0.09	0.17	-0.35	-0.01
Bill depth	-0.82	-0.33	0.05	0.17	0.43	0.04
Body mass	-0.94	0.12	-0.16	-0.13	0.03	-0.25
Wing chord	-0.86	-0.01	-0.36	-0.33	-0.05	0.16
Tarsus length	-0.74	0.52	-0.11	0.40	-0.08	0.06
Standard deviation	1.99	0.86	0.70	0.63	0.57	0.30
Proportion of variance	0.66	0.12	0.08	0.07	0.05	0.02
Cumulative proportion	0.66	0.78	0.87	0.93	0.98	1
PCA model λ			0.87			

Table 4.1 Principal component scores from phylogenetic PCA on morphological measurements of N=96 species.

	PC1- Size	PC2-Tarsus vs. Bill Shape	PC3-Bill Length	PC4	PC5	PC6
Bill length	-0.76	0.24	0.56	-0.21	-0.03	0.04
Bill width	-0.74	-0.54	0.09	0.17	-0.35	-0.01
Bill depth	-0.82	-0.33	0.05	0.17	0.43	0.04
Body mass	-0.94	0.12	-0.16	-0.13	0.03	-0.25
Wing chord	-0.86	-0.01	-0.36	-0.33	-0.05	0.16
Tarsus length	-0.74	0.52	-0.11	0.40	-0.08	0.06
Standard deviation	1.99	0.86	0.70	0.63	0.57	0.30
Proportion of variance	0.66	0.12	0.08	0.07	0.05	0.02
Cumulative proportion	0.66	0.78	0.87	0.93	0.98	1
PCA model λ			0.87			

Table 4.2 Principal component scores from phylogenetic PCA on animal, fruit and seed diet scores of N=96 species.

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CHAPTER 5: PRESSURE FOR RAPID AND ACCURATE MATE RECOGNITION PROMOTES AVIAN-PERCEIVED PLUMAGE SEXUAL DICHROMATISM IN TRUE THRUSHES

Abstract

Ecological conditions limiting the time to find a compatible mate or increasing the difficulty in doing so likely promote the evolution of traits used for species and mate recognition. Conspicuous traits that signal an individual's species, sex, and breeding status reduce the challenge of identifying a compatible conspecific mate and should be more common in migratory rather than sedentary species, species with shorter breeding seasons, and species breeding under high sympatry with many closely-related heterospecifics. Here, we tested this recognition hypothesis for promoting plumage sexual dichromatism in the true thrushes (*Turdus* spp.), a large and diverse genus of passerine birds. We used receptor-noise limited models of avian vision to quantify avian-perceived chromatic and achromatic visual contrasts between male and female plumage patches and tested the influence of breeding season length, spatial distribution, and sympatry with other *Turdus* species on plumage dichromatism. As predicted, we found that 1) true thrush species with migratory behavior have greater plumage sexual dichromatism than non-migratory species, 2) species with longer breeding seasons have less plumage sexual dichromatism, and 3) greater numbers of *Turdus* thrush species breeding in sympatry is associated with more plumage sexual

dichromatism. These results suggest that social recognition systems, including species and mate recognition, play a prominent role in the evolution of plumage sexual dichromatism in true thrushes.

Introduction

Species recognition is necessary in sexually reproducing lineages for individuals to find compatible mates and produce viable offspring (Andersson 1994; Gröning and Hochkirch 2008). Conspicuous traits signaling species and sex identity increase the ease and speed of mate recognition by reducing the effort, error, and time involved when searching for compatible mates and lessen the likelihood of mating with heterospecifics (Pfennig and Hurlbert 2012). Traits used in species and mate recognition may also serve as signals of status to conspecifics and reduce costly conflicts over resources and mates (West-Eberhard 1983). Accordingly, distinct traits facilitating mate recognition should be more likely to arise and be maintained under conditions that increase both the difficulty of finding a compatible mate and degree of resource competition among conspecifics and closely-related species. Conditions likely to favor traits signaling an individual's species, sex, and breeding status include higher sympatry with many closely-related species, limited time to find compatible breeding mates, and lower rates of encounter with potential breeding mates (Andersson 1994).

In birds, plumage color is a highly conspicuous trait signaling species and (often) sex identity (Martin et al. 2015; Bitton and Doucet 2016). Plumage sexual dichromatism, or the distinct set of differences in the appearance of male and female feather colors and patterns, is common in birds and is usually attributed to different natural and sexual selection pressures on males and females (Martin and Badyaev 1996; Burns 1998; Badyaev and Hill 2003; Dale et al. 2015; Dunn et al. 2015). Plumage sexual dichromatism results in a visibly perceivable trait useful for recognizing an individual's species, sex, and breeding status (e.g., in species with sex-specific delayed plumage maturation, see Hawkins et al. 2012), reducing the time and effort expended to identify a suitable mate (Hamilton 1961; Saetre and Slagsvold 1992). Evidence in favor of this recognition hypothesis for sexual dichromatism in birds includes a positive association of greater plumage sexual dichromatism with migratory behavior and shorter breeding seasons (Badyaev and Hill 2003), both of which reduce the amount of time available to search and find suitable mates and successfully breed. Additional support for the recognition hypothesis includes a consistent pattern of greater plumage sexual dichromatism and plumage color elaboration in avian species that reside on mainland continents and have large geographic ranges in comparison to species that do not migrate, reside on islands, and have limited breeding ranges (Badyaev and Ghalambor 1998; Figuerola and Green 2000; Friedman et al. 2009; Tobias and Seddon 2009; Roulin and Salamin 2010; Dale et

al. 2015; Simpson et al. 2015; Doutrelant et al. 2016; Matysioková et al. 2017; Kearns et al. 2020).

Moreover, plumage sexual dichromatism likely plays a role in hybridization avoidance via reproductive character displacement to facilitate species and mate recognition, especially among closely-related species. For example, in *Ficedula* flycatchers, female choice selects for divergent male plumage coloration across populations and species, leading to male character displacement and reduced rates of interspecific hybridization (Alatalo et al. 1994; Saetre et al. 1997; Laaksonen et al. 2015). More broadly and across taxa, greater plumage dichromatism is positively associated with higher breeding sympatry with closely-related heterospecifics. Among a large sample of passerine sister species pairs, transitions from allopatry to parapatry and increases in geographic range overlaps are positively correlated with greater plumage dichromatism (Cooney et al. 2017). Greater plumage sexual dichromatism has also been found to be positively associated with greater avian species divergence and richness (Seddon et al. 2013; Cooney et al. 2019). Among passerine sister species pairs, more pronounced changes in male rather than female plumage coloration in sexually-dichromatic species suggest that female choice and male-male competition often lead to concurrent increases in sexual dichromatism and speciation events (Seddon et al. 2013). Therefore, plumage sexual dichromatism may be a selected trait for facilitating species

and mate recognition when closely-related species have sympatric breeding ranges (Martin et al. 2010, 2015).

True thrushes (*Turdus* spp.) are an exceptionally diverse monophyletic genus of passerine birds consisting of about ~86 species distributed across the globe (Fig. 5.1). The true thrushes are an ideal passerine clade for examining the recognition hypothesis for plumage sexual dichromatism because plumage sexual dichromatism and migratory behaviors vary substantially between species and sexual dichromatism has evolved multiple times in thrushes across the world (Clement and Hathway 2000; Nagy et al. 2019). Hybridization also occurs in some, but not all, *Turdus* species, indicating that some sympatric *Turdus* species can successfully interbreed. A particularly well-documented example of hybridization in true thrushes occurs at a large hybrid zone between four *Turdus* species (*T. atrogularis*, *T. eunomus*, *T. naumanni*, *T. ruficollis*) in north-central Asia (McCarthy 2006). Further, plumage sexual dichromatism in true thrushes often coincides with age and breeding status in male thrushes. Delayed plumage maturation in males is common among true thrushes (Escalona-Segura and Peterson 1997; Peterson et al. 2003; Ligon and Hill 2013), where males have “female-like” plumage coloration during their first breeding season and develop typical breeding adult male plumage for subsequent breeding seasons. The presence of delayed plumage maturation and distinct juvenile plumage may serve as a signal of a young

male's sexual immaturity in order to reduce levels conspecific aggression from older adults (Ligon and Hill 2013) and also suggests that female thrushes prefer older males with prominent adult plumage as breeding mates.

Overall, ecological conditions that increase the time and degree of difficulty in finding a suitable conspecific mate should select for phenotypic traits that reliably signal species and sex identity. Across various bird lineages, greater plumage dichromatism is present in species that are i) migratory rather than nonmigratory, ii) have shorter breeding seasons, iii) live on mainlands rather than islands, iv) have larger breeding ranges (distributions), and v) breed in sympatry with more closely-related species. These patterns suggest that ecological circumstances where rapid and accurate mate recognition is challenging strongly favor the evolution and maintenance of prominent plumage sexual dichromatism in birds. Here, we test these predictions of the recognition hypothesis for plumage sexual dichromatism by evaluating the potential influences of breeding timing, spacing, and sympatry on plumage dichromatism in *Turdus* thrushes (Fig. 5.2).

Methods

Initial pre-registration of the study's methods and analyses are available on Open Science Framework (Luro and Hauber 2019).

Plumage sexual dichromatism

A total of $N=77$ *Turdus* thrush species (approximately ~89% of all known true thrush species) were sampled for plumage spectral reflectance using prepared bird skin specimens at the American Museum of Natural History in New York City and the Field Museum in Chicago, USA. Reflectance measurements spanning 300-700nm were taken in triplicate from the belly, breast, throat, crown, and mantle plumage patches (Andersson and Prager 2006) of each individual. $N=3$ male and $N=3$ female individuals were measured for most species (exceptions: *T. lawrencii*, $N=2$ males and $N=2$ females; *T. swalesi*, $N=1$ male and $N=1$ female). Reflectance spectra were measured using a 400 μm fiber optic reflection probe fitted with a rubber stopper to maintain a consistent measuring distance of 3 mm and area of 2 mm^2 at a 90° angle to the surface of the feather patch. Measurements were taken using a JAZ spectrometer with a pulsed-xenon light source (Ocean Optics, Dunedin, USA), and we used a diffuse 99% reflectance white standard (Spectralon WS-1-SL, Labsphere, North Sutton NH, USA).

We applied a receptor-noise limited visual model (Vorobyev and Osorio 1998) of the European Blackbird (*T. merula*) visual system (Hart et al. 2000) in the *pavo* (Maia et al. 2019 p. 2) package in R v4.0.0 (R Core Team 2020) to calculate avian-perceived chromatic and achromatic visual contrast (in units of “Just-Noticeable Differences,” or JNDs) of male vs. female plumage patches for all sampled *Turdus* species. Chromatic

and achromatic JNDs were calculated for male-female pairs within each species (i.e., $N=9$ JND values calculated per patch for each species where $N=3$ males and $N=3$ females sampled), and then JND values were averaged for each species' respective plumage patches. Under ideal laboratory conditions, 1 JND is generally considered to be the discriminable threshold past which an observer is predicted to be able to perceive two colors as different. However, natural light environments vary both spatially and temporally (Endler 1993), bringing into question the accuracy of a 1 JND threshold for generalizing visual contrast under natural conditions. Therefore, we calculated the total number of sexually-dichromatic plumage patches per species (out of $N=5$ measured patches) as the number of plumage patches with average JND values > 1 , 2 , or 3 to account for uncertainty in visual discrimination thresholds due to variation in psychophysical and ambient lighting conditions affecting the strength of between-sex plumage visual contrast (Kemp et al. 2015). Additionally, we modeled the number of divergent plumage patches (at the three different JND thresholds listed above) within sexes and between different sympatric species under different levels of breeding range overlap (10% increments between 0-90%).

Life history data

Breeding timing model

We collected data on migration behavior and breeding season length from *Thrushes* (Clement and Hathway 2000) and the *Handbook of the Birds of the World* (del Hoyo et al. 2017). We assigned three different kinds of migratory behavior: 1) *full migration* when a species description clearly stated that a species “migrates,” 2) *partial migration* when a species was described to have “altitudinal migration,” “latitudinal migration” or “movement during non-breeding season,” or 3) *sedentary* when a species was described as “resident” or “sedentary.” Breeding season length was defined as the number of months the species breeds each year.

Breeding sympatry model

Species breeding ranges were acquired from *BirdLife International* (BirdLife International and Handbook of the Birds of the World 2018). We calculated congener breeding range overlaps (as percentages) using the *letsR* package in R (Vilela and Villalobos 2015). We then calculated the number of sympatric species as the number of congeners with breeding ranges that overlap >30% with the focal species breeding range (Cooney et al. 2017). Comparisons of the number of sexually-dimorphic plumage patches vs. the number of sympatric species among different breeding range overlap thresholds are provided in Figure 5.4.

Breeding spacing model

Species breeding range sizes (in km²) were acquired using the *BirdLife International* breeding range maps. Species island vs. mainland residence was also determined using breeding ranges from *BirdLife International*. Mainland residence was assigned if the species had a breeding range on any continent and Japan. Island residence was assigned to species having a breeding range limited to a non-continental landmass entirely surrounded by a marine body of water.

Statistical modeling

We used phylogenetically-corrected Bayesian multilevel logistic regression models using the *brms* v2.13.0 package (Bürkner 2017) in R v4.0.0 (R Core Team 2020). We modeled plumage sexual dichromatism responses as the number of sexually-dichromatic patches > 1, 2, or 3 chromatic and achromatic JNDs. Plumage dichromatism responses were modeled as binomial trials (N=5 plumage patch “trials”) to test for associations with breeding timing, breeding sympatry and breeding spacing. For all phylogenetically-corrected models, we used the *Turdus* molecular phylogeny from Nylander et al. (2008) to create a covariance matrix of species phylogenetic relationships. All models used a dataset of N=67 out of the *Turdus* species for which all the types of data (see above) were available.

Our *breeding timing* models included the following predictors: z-scores of breeding season length (mean-centered by $\mu = 5.4$ months, and scaled by one standard deviation $\sigma = 2.3$ months), migratory behavior (no migration as the reference category versus partial or full migration), and their interaction. *Breeding sympatry* models included the number of sympatric species with greater than 30% breeding range overlap as the only predictor of the probability of having a sexually-dichromatic plumage patch. *Breeding spacing* models included $\log_e$ transformed breeding range size (km²) and breeding landmass (mainland as the reference category versus island). We also ran null models (intercept only) for all responses. All model intercepts and response standard deviations were assigned a weakly informative prior (Student T: df = 3, location = 0, scale = 10) (Gelman et al. 2013), and predictor coefficients were assigned flat uninformative priors. We ran each model for 6,000 iterations across 6 chains and assessed Markov Chain Monte Carlo (MCMC) convergence using the Gelman-Rubin diagnostic (Rhat) (Gelman et al. 2013). We then performed k-fold cross-validation (Vehtari et al. 2017) to refit each model $K=16$ times. For each k-fold, the training dataset included a randomly selected set of $N \approx 63$ species, and the testing dataset included $N \approx 4$ species not included in the training dataset. Finally, we compared differences between the models' expected log pointwise predictive densities (ELPD) to assess which

model(s) best predicted the probability of having a sexually-dichromatic plumage patch. (Vehtari et al. 2017).

Model predictor effects were assessed using 90% highest-density intervals of the posterior distributions and probability of direction, the proportion of the posterior distribution that shares the same sign (positive or negative) as the posterior median (Makowski et al. 2019), to provide estimates of the probability that a predictor has an entirely positive or negative effect on the presence of sexually-dimorphic plumage patches. We assume predictor estimates with a probability of direction ≥ 0.90 to be indicative of a reliable existence of a predictor effect on sexually-dimorphic plumage patches (Makowski et al. 2019).

Results

Avian visual modeling

Among N=77 *Turdus* species, the following proportion have sexually monomorphic plumage (combined achromatic and chromatic JND thresholds): 1.3% (n=1 species) have no sexually-dimorphic patches > 1 JND, 44% (n=34 species) have no dimorphic patches > 2 JND, and 63% (n=49 species) have no dimorphic patches > 3 JND (Table 5.3). Additional proportions of *Turdus* species with sexually-dimorphic achromatic or chromatic plumage patches are available in Table 5.4. When comparing within sexes between sympatric species (i.e., following Cooney et al. (2017) at least a

30% overlap in breeding ranges: $n=39$ species with at least one sympatric species and a median of $n=6$ sympatric species per focal species), the median number of avian-discriminable plumage patches between species is 1 or greater for all three achromatic and chromatic JND thresholds except for sympatric females at a chromatic JND threshold > 3 (Fig. 5.3).

Model comparisons

Breeding sympatry, *breeding timing*, and *breeding spacing* performed considerably better than *intercept-only* (null models) in predicting the probability of a species having a sexually-dimorphic plumage patch. We obtained $N \geq 4000$ effective posterior samples for each model parameter and all model Markov Chains (MCMC) successfully converged ($R_{hat} = 1$ for all model parameters). All *breeding sympatry*, *breeding timing*, and *breeding spacing* models performed similarly well and substantially better than *intercept only* models in predicting the probability of having a sexually-dimorphic plumage patch with achromatic JND values $> 1, 2$, or 3 (Table 5.1; all models predicting achromatic plumage patches had ELPD values within 4, following the convention of Burnham and Anderson (2002)). Among models predicting the probability of having a sexually-dichromatic plumage patch with chromatic JND values $>1, 2$, or 3 , all *breeding sympatry*, *breeding timing*, and *breeding spacing* models performed much better than *intercept only* models, and *breeding sympatry* models had the top predictive performance

(Table 5.1; *breeding sympatry* models all have $ELPD = 0$, only the *breeding spacing* models predicting dichromatic plumage patches had similar predictive performance).

Achromatic plumage sexual dichromatism predictors

Migratory behavior and shorter breeding season lengths were strongly associated with greater odds of a species having achromatic plumage sexual dichromatism. All model predictor effect estimates are provided as the posterior median odds-ratio (OR) and 90% highest-density interval (HDI) in Table 5.2. Among predictors of achromatic sexually-dimorphic plumage patches, only predictors included in the *breeding timing* model have predictors with probability of direction (pd) values ≥ 0.90 (Table 5.2). Specifically, longer breeding season length was associated with lower odds of a species having a sexually-dimorphic plumage patch with achromatic $JND > 2$ (breeding season length, OR [90% HDI] = 0.10 [0.01, 1.1], 89.5% decrease in odds per 2.3-month increase in breeding season) and $JND > 3$ (breeding season length, OR [90% HDI] = 0.25 [0.03, 1.5], 75% decrease in odds per 2.3-month increase in breeding season). Additionally, full migratory behavior, rather than no migratory behavior, was associated with greater odds of a species having a sexually-dimorphic plumage patch with achromatic $JND > 1$ (full migration, OR [90% HDI] = 4.97 [0.95, 24.4]), $JND > 2$ (full migration, OR [90% HDI] = 66.5 [3.2, 1802.4]) and $JND > 3$ (OR [90% HDI] = 22.3 [1.6, 307.9]). Finally, both full and partial migratory behavior, rather than no migration

behavior, in conjunction with longer breeding season lengths are associated with greater odds of a species having a sexually-dimorphic plumage patch with achromatic JND > 1 (breeding season length x full migration, OR [90% HDI] = 4.84 [0.67, 39.6]), JND > 2 (breeding season length x full migration, OR = 66.3 [0.59, 11415.7]; breeding season length x partial migration, OR [90% HDI] = 20.7 [0.9, 589.1]) and JND > 3 (breeding season length x partial migration, OR [90% HDI] = 8.28 [0.76, 109.1]).

Chromatic plumage sexual dichromatism predictors

Migratory behavior, shorter breeding season lengths, and larger numbers of sympatric *Turdus* species were strongly associated with greater odds of a species having chromatic plumage sexual dichromatism. Among predictors of *breeding timing* models predicting chromatic sexually-dimorphic plumage patches, longer breeding season length was associated with lower odds of a species having a plumage patch with chromatic JND > 2 (OR [90% HDI] = 0.14 [0.01, 1.42], 86% reduction in odds per 2.3 month increase in breeding season). Both full and partial migratory behavior rather than no migration are associated with greater odds of a species having a plumage patch JND > 1 (partial migration, OR [90% HDI] = 2.2 [0.94, 4.9]), JND > 2 (full migration, OR [90% HDI] = 80.51 [2.8, 3432.9]) and JND > 3 (partial migration, OR [90% HDI] = 71.2 [0.32, 59062.9]; full migration, OR [90% HDI] = 234.7 [0.51, 300382.6]). For *breeding spacing models*, island residency rather than mainland residency was associated with

lower odds of having a plumage patch > 1 chromatic JND (island, OR [90% HDI] = 0.27 [0.09, 0.89]). Finally, more *Turdus* species in sympatry were associated with higher odds of a species having a sexually-dimorphic chromatic plumage patch with JND > 1 (number of sympatric species, OR [90% HDI] = 1.4 [1.18, 1.67], 40% increase in odds per each additional sympatric species), JND > 2 (sympatric species, OR [90% HDI] = 1.59 [1.01, 2.52], 59% increase in odds per each additional sympatric species), and JND > 3 (sympatric species, OR [90% HDI] = 2.11 [1.03, 4.46], 111% increase in odds per each additional sympatric species).

Discussion

Our results provide comparative correlative evidence in support of predictions of the recognition hypothesis for plumage sexual dichromatism in true thrushes. We used a receptor-noise limited model of *Turdus merula* vision (Vorobyev and Osorio 1998; Hart et al. 2000) to measure avian-perceivable visual contrast of plumage colors and found that the odds of plumage sexual dichromatism are much greater for *Turdus* thrush species that have full or partial migration rather than no migration, have relatively short breeding seasons, and are in sympatry with many other true thrush species (Table 5.1, 5.2). Our results align with prior comparative studies of avian plumage sexual dichromatism where strong associations of sexual dichromatism with

greater migratory behavior (Dale et al. 2015) and more sympatric taxa (Cooney et al. 2017) were found among many species of different passerine families.

Further, we determined that sympatric *Turdus* species have distinguishable plumage coloration differences from one another when measuring plumage appearance from the avian visual perspective (Fig. 5.3). Divergent plumage coloration within sexes between closely-related species indicates that plumage sexual dichromatism may have evolved to facilitate species and mate recognition in *Turdus* species breeding under higher sympatry with other true thrushes. However, we cannot directly determine if the plumage sexual dichromatism in sympatric *Turdus* species is the result of reproductive character displacement. We do not know if past changes in species plumage sexual dichromatism occurred before or during periods of sympatry with other *Turdus* species. Regardless, present-day plumage sexual dichromatism and perceivable differences in plumage coloration between sympatric species likely reduces the challenge of finding compatible mates by signaling an individual's sex, breeding status, and species. For example, the four species *Turdus* hybrid zone in north-central Asia (McCarthy 2006) is a particularly striking example where reproductive character displacement has likely occurred and all four species exhibit strong plumage sexual dichromatism (Fig. 5.5). Comparing within sexes between sister species pairs of *T.ruficollis* and *T.atrogularis*, and *T.naumanni* and *T.eunomus* (Nylander et al. 2008), plumage patterns of the species pairs

are nearly identical except for a divergence in color. *T.ruficollis* and *T.atrogularis* share similar facial and throat coloring patterns, with the main difference being red coloration in *T.ruficollis* in opposition to the black coloration of *T.atrogularis*. In the second species pair, *T.naumanni* has red ventral plumage coloration and *T.eunomus* has black ventral plumage coloration.

Previous studies have found that closely-related sympatric species tend to have more similar plumage appearance than expected if plumage coloration patterns had evolved to facilitate species recognition via reproductive character displacement (Miller et al. 2019; Simpson et al. 2021). The potential lack of major plumage color divergence among closely-related sympatric species may be attributable to constraints imposed by a shared light environment on color signal efficiency (McNaught and Owens 2002), or similar natural selection pressures (e.g., predators, parasites, and weather). Generally, despite greater similarity in plumage appearance in comparison to allopatric species, closely-related sympatric species can still have substantially different and biologically-relevant differences in achromatic or chromatic interspecific visual contrast of plumage patches when measuring plumage coloration differences from the avian visual perspective (as we have found in our analyses).

Patterns of plumage sexual dichromatism in true thrushes (*Turdus*) are consistent with select predictions of the recognition hypothesis for plumage sexual dichromatism.

Migratory behavior and limited breeding seasons reduce the amount of time available to find a mate, and greater plumage sexual dichromatism may help migratory species find compatible mates more rapidly. Greater plumage sexual dichromatism in *Turdus* species under sympatry with other true thrush species also supports the possibility that increased plumage sexual dichromatism may be the result of reproductive character displacement. Therefore, greater plumage sexual dichromatism likely increases the speed and accuracy of finding a compatible breeding mate, reduces species and mate recognition errors, and decreases hybridization.

Figures and Tables

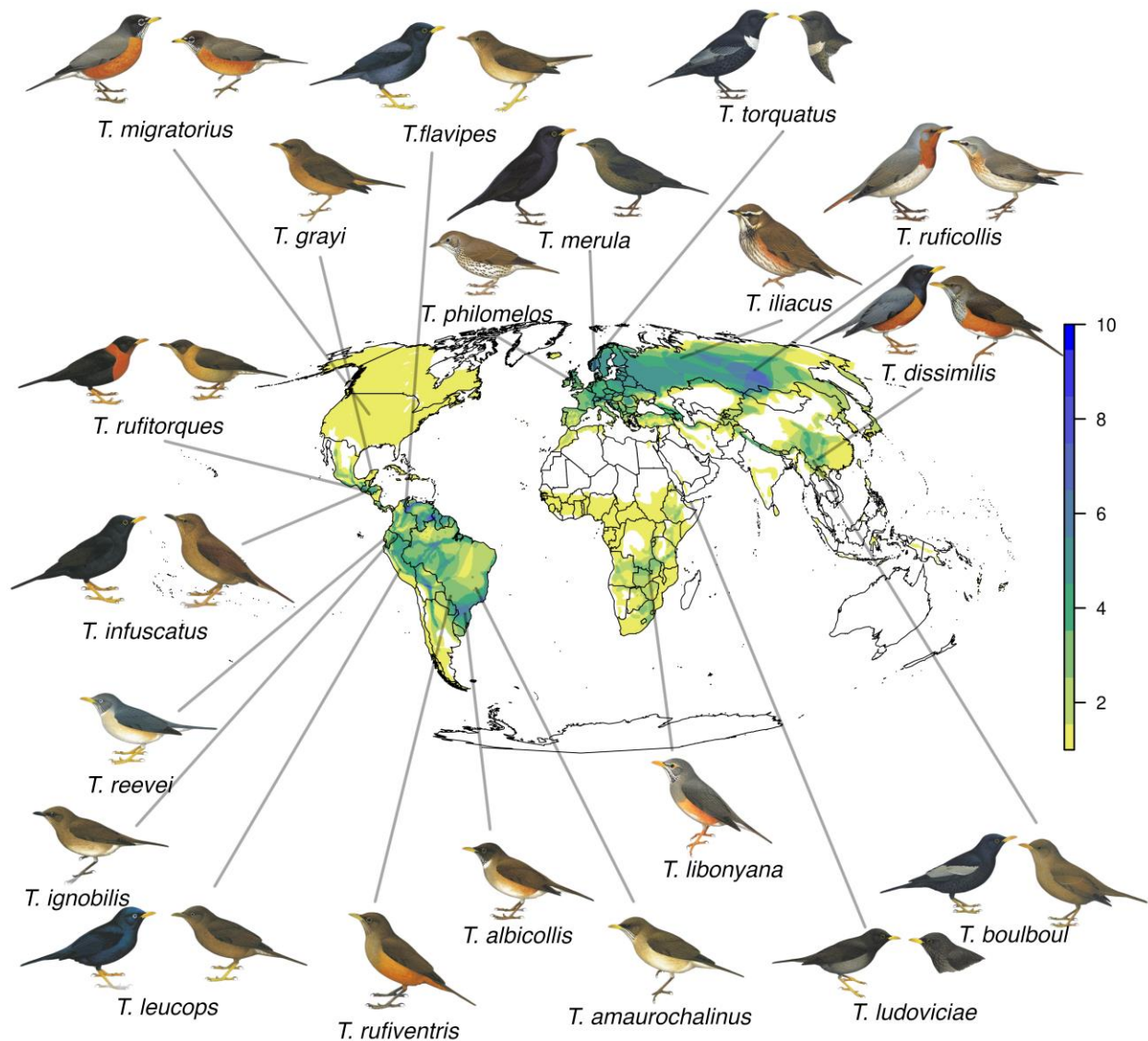


Figure 5.1 Breeding ranges of all recognized *Turdus* species from BirdLife International, with representative species' males and females shown for species with sexually dimorphic plumage. The color scale indicates the number of *Turdus* thrush species in sympatry with overlapping breeding ranges. Illustrations used with permission from HBW Alive/Lynx Edicions.

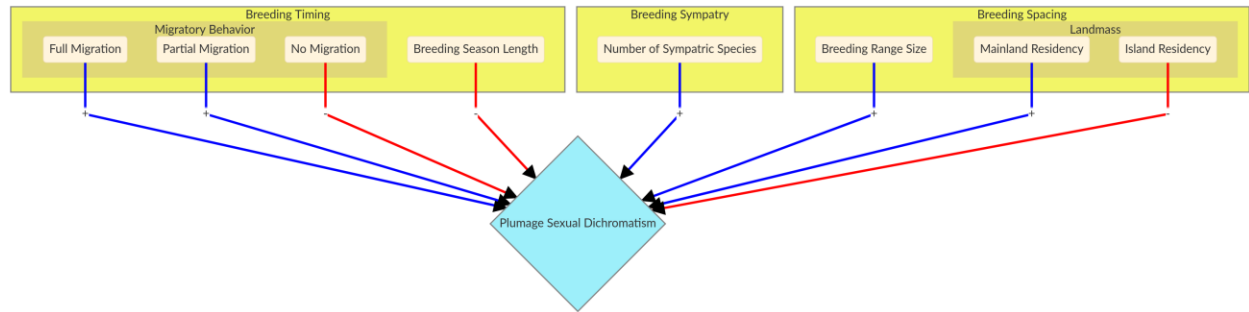


Figure 5.2 Hypotheses and predictions for each model (large yellow boxes). Arrow colors indicate predicted correlation, positive (blue) and negative (red).

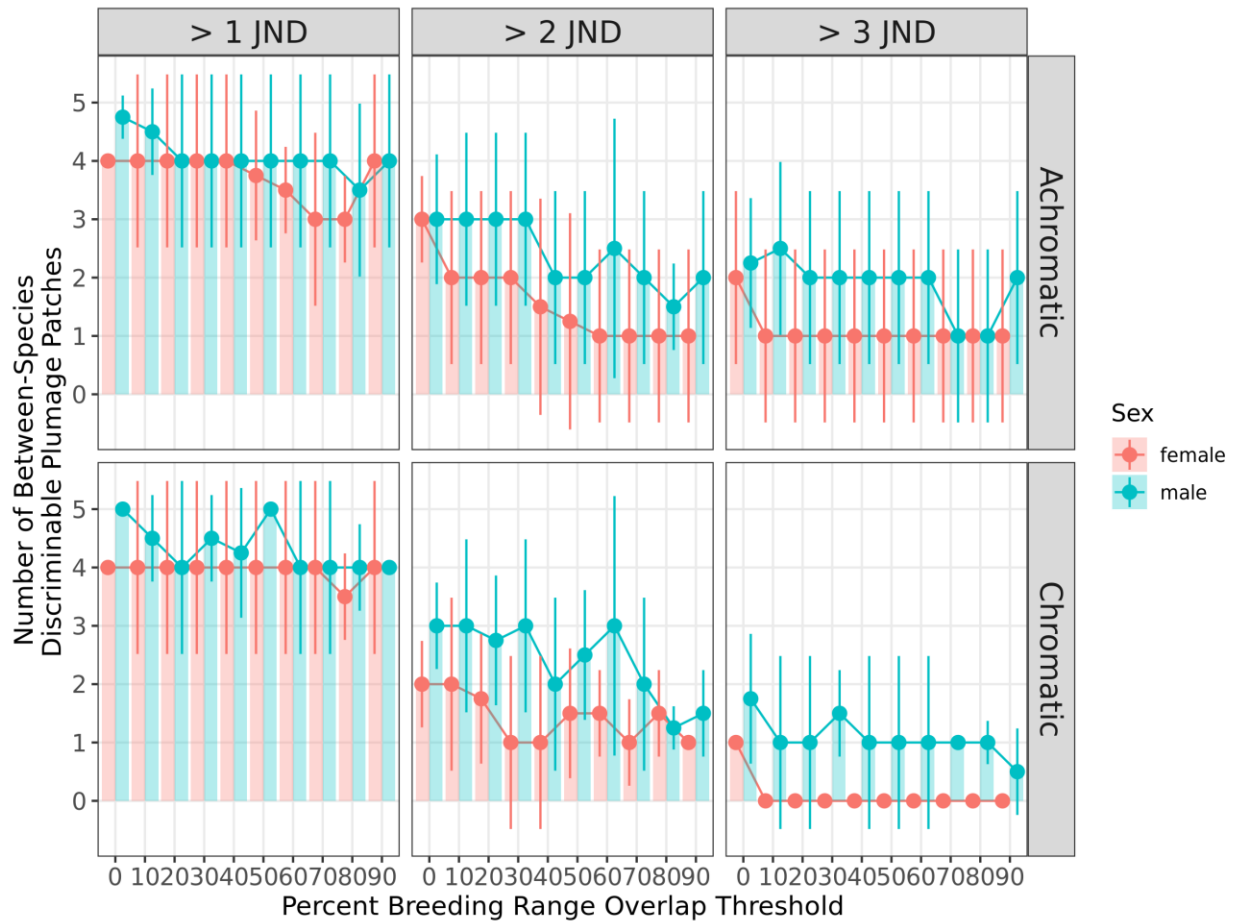


Figure 5.3 Median $\pm$ median absolute deviation of number of distinguishable plumage patches by just noticeable difference (JND) thresholds of 1, 2, and 3 between male and female *Turdus* thrush species in sympatry at various breeding range overlaps (percent).

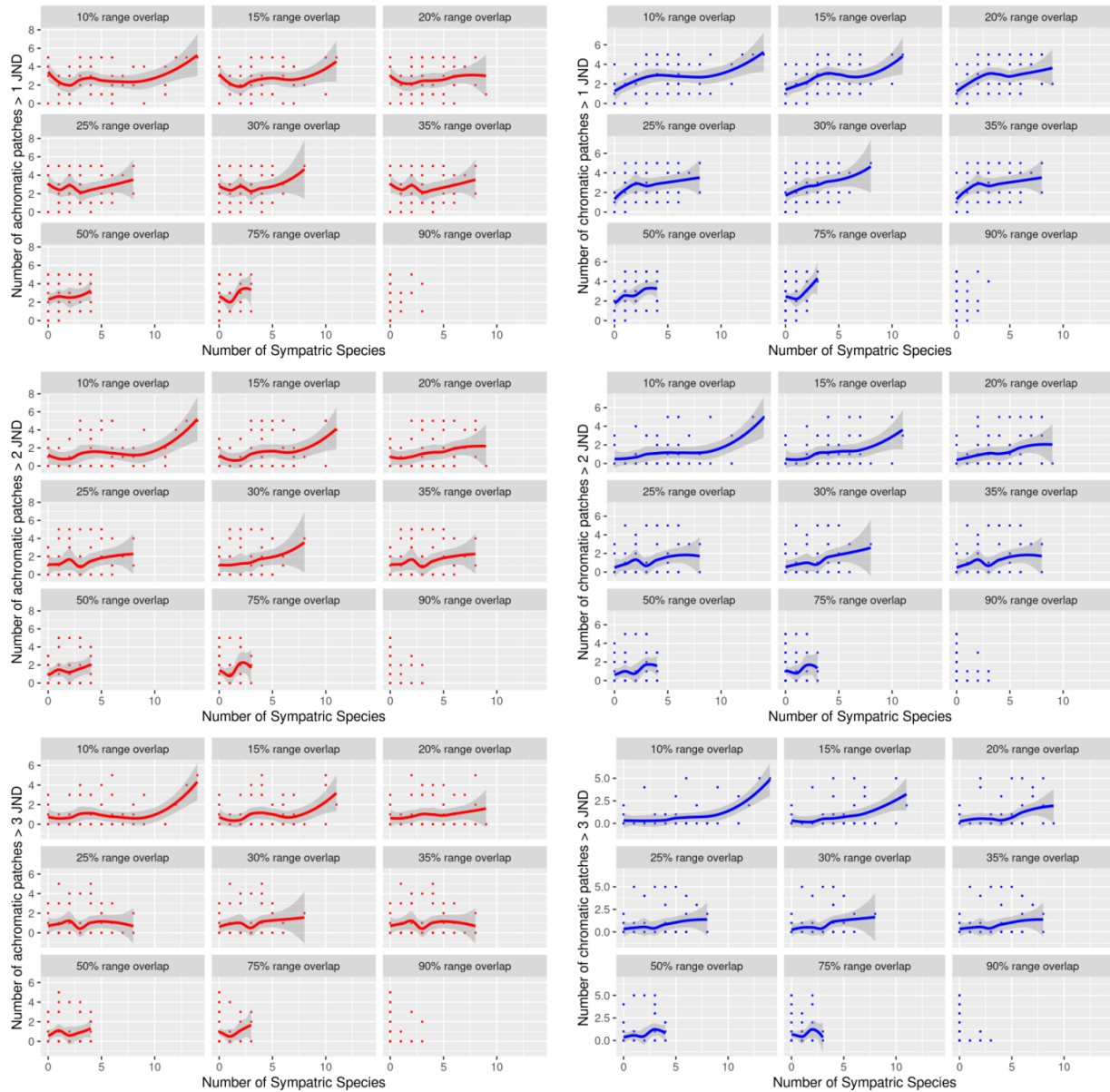


Figure 5.4 Number of sexually-dichromatic chromatic and achromatic plumage patches versus number of sympatric *Turdus* species, faceted by sympatry overlap thresholds (0-90%). Lines are Loess nonlinear regression fits with no correction for phylogenetic relatedness among species.

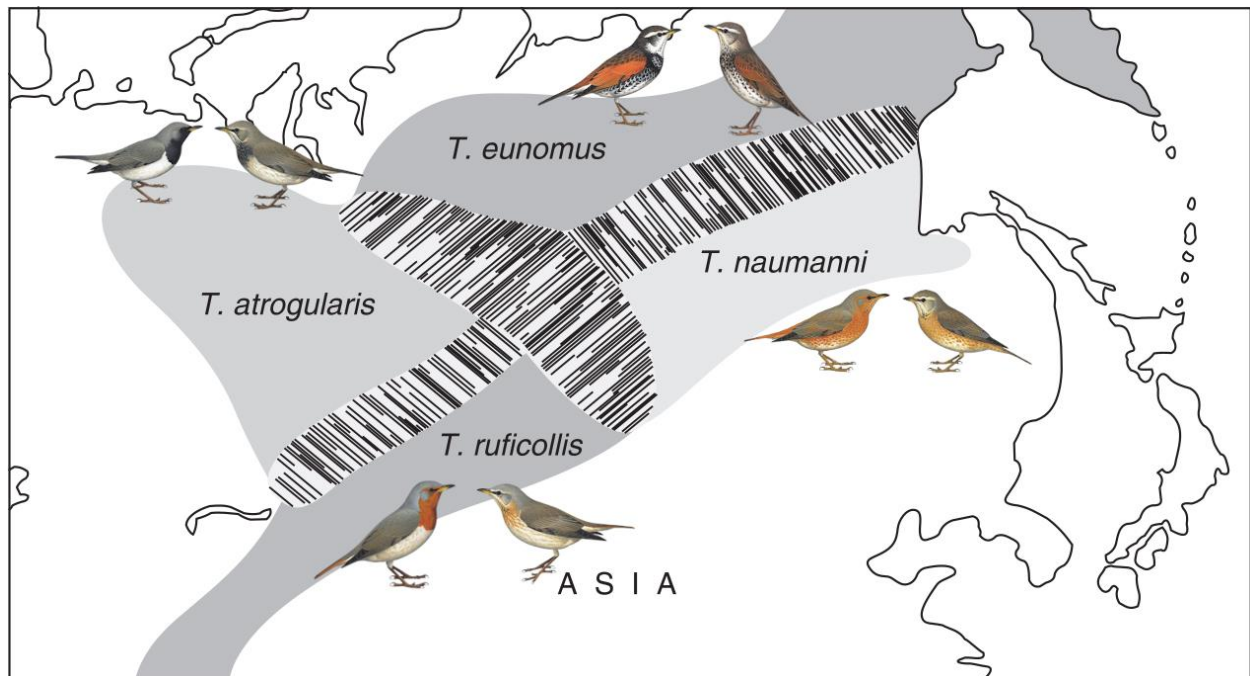


Figure 5.5 Four species hybrid zone in north-central Asia (*T.atrogularis*, *T.ruficollis*, *T.eunomus*, and *T.naumanni*). Map is from (McCarthy 2006). Illustrations © HBW Alive/Lynx Edicions.

Plumage Metric	JND Threshold	Model			
		Breeding Sympatry	Breeding Timing	Breeding Spacing	Intercept Only
Achromatic					
	1 JND	0 ± 0 (-122.17 ± 0.67)	-2.51 ± 2.49 (-124.68 ± 2.38)	-2.59 ± 1.01 (-124.76 ± 1.04)	-21.69 ± 7.36 (-143.87 ± 7.51)
	2 JND	0 ± 0 (-98.94 ± 7.56)	-1.19 ± 3.95 (-100.13 ± 9.22)	-0.7 ± 1.34 (-99.64 ± 7.92)	-52.42 ± 12.67 (-151.36 ± 13.4)
	3 JND	-0.04 ± 1.4 (-85.4 ± 8.91)	-1.7 ± 4.41 (-87.07 ± 10.71)	0 ± 0 (-85.37 ± 8.76)	-28.54 ± 10.02 (-113.91 ± 13.65)
Chromatic					
	1 JND	0 ± 0 (-115.75 ± 2.95)	-5.67 ± 3.55 (-121.42 ± 2.28)	-2.73 ± 3.4 (-118.49 ± 2.67)	-14.8 ± 7.22 (-130.55 ± 7.05)
	2 JND	0 ± 0 (-88.47 ± 8.77)	-3.8 ± 4.46 (-92.27 ± 10.01)	-3.32 ± 5.29 (-91.79 ± 10.91)	-50.53 ± 14.49 (-139 ± 16.77)
	3 JND	0 ± 0 (-62.77 ± 10.41)	-8 ± 4.32 (-70.77 ± 12.29)	-4.43 ± 3.9 (-67.2 ± 11.72)	-47.63 ± 15.34 (-110.4 ± 20.01)

Table 5.1 Expected log pointwise predictive density (ELPD) differences and kfold information criterion values of models (ELPD Difference ± standard error (kfold IC ± standard error)). Values closest to zero indicate greater model accuracy for predicting species plumage patch dichromatism.

Model	Parameter	Achromatic, JND > 1	Achromatic, JND > 2	Achromatic, JND > 3	Chromatic, JND > 1	Chromatic, JND > 2	Chromatic, JND > 3
Breeding Timing	Intercept	0.31 (0.02, 5.29), pd = 0.76	0 (0, 0.54), pd = 0.98	0 (0, 0.19), pd = 0.99	0.41 (0.05, 2.79), pd = 0.78	0 (0, 1.73), pd = 0.95	0 (0, 1.37), pd = 0.96
	Breeding Season Length	0.94 (0.54, 1.75), pd = 0.57	0.1 (0.01, 1.05), pd = 0.97	0.25 (0.03, 1.49), pd = 0.91	0.89 (0.56, 1.4), pd = 0.66	0.14 (0.01, 1.42), pd = 0.94	0.08 (0, 9.14), pd = 0.83
	Partial Migration vs. No Migration	0.96 (0.31, 2.75), pd = 0.53	4.11 (0.3, 61.54), pd = 0.83	3.65 (0.44, 35.64), pd = 0.85	2.2 (0.94, 4.89), pd = 0.94	6.7 (0.42, 134.8), pd = 0.88	71.16 (0.32, 59062.92), pd = 0.92
	Full Migration vs. No Migration	4.97 (0.95, 24.41), pd = 0.96	66.52 (3.19, 1802.4), pd = 0.99	22.34 (1.59, 307.91), pd = 0.98	2.29 (0.69, 7.31), pd = 0.88	80.51 (2.81, 3432.88), pd = 0.99	234.71 (0.51, 300382.62), pd = 0.95
	Breeding Season Length x Partial Migration	1.34 (0.48, 3.92), pd = 0.68	20.71 (0.87, 589.09), pd = 0.96	8.28 (0.76, 109.11), pd = 0.94	1.39 (0.65, 3.12), pd = 0.76	9.03 (0.44, 251.36), pd = 0.9	34.46 (0.08, 68228.71), pd = 0.85
	Breeding Season Length x Full Migration	4.84 (0.67, 39.63), pd = 0.9	66.3 (0.59, 11415.7), pd = 0.93	16.41 (0.27, 824.69), pd = 0.89	1.68 (0.31, 8.33), pd = 0.7	160.6 (0.84, 67791.13), pd = 0.95	433.67 (0.01, 37194569.46), pd = 0.85
	Phylogenetic Signal λ , Median (90% Credible Interval)	0.29 (0.16, 0.43)	0.72 (0.56, 0.86)	0.61 (0.42, 0.8)	0.17 (0.08, 0.28)	0.74 (0.57, 0.88)	0.89 (0.77, 0.97)
Breeding Spacing	Intercept	0.14 (0, 7.49), pd = 0.8	0 (0, 2.44), pd = 0.95	0 (0, 0.14), pd = 0.98	0.51 (0.03, 9.7), pd = 0.65	0 (0, 7.63), pd = 0.92	0 (0, 81.95), pd = 0.91
	Island vs. Mainland	1.08 (0.25, 4.79), pd = 0.54	0.53 (0.01, 17.83), pd = 0.61	0.92 (0.05, 19.32), pd = 0.52	0.27 (0.09, 0.89), pd = 0.97	0.03 (0, 3.99), pd = 0.89	0.04 (0, 67.59), pd = 0.77
	Breeding Range Size	1.08 (0.88, 1.32), pd = 0.75	1.23 (0.76, 2.01), pd = 0.77	1.3 (0.87, 1.93), pd = 0.87	1.02 (0.87, 1.19), pd = 0.58	1.24 (0.75, 2.05), pd = 0.77	1.26 (0.54, 2.99), pd = 0.69
	Phylogenetic Signal λ , Median (90% Credible Interval)	0.27 (0.15, 0.41)	0.71 (0.56, 0.85)	0.6 (0.42, 0.77)	0.15 (0.07, 0.25)	0.72 (0.55, 0.86)	0.85 (0.71, 0.95)
Breeding Sympatry	Intercept	0.41 (0.03, 5.83), pd = 0.72	0 (0, 0.98), pd = 0.95	0 (0, 0.34), pd = 0.98	0.25 (0.04, 1.35), pd = 0.91	0 (0, 1.12), pd = 0.95	0 (0, 0.29), pd = 0.98
	Number of Sympatric Species ($\geq$ 30% Breeding Range Overlap)	1.03 (0.84, 1.27), pd = 0.61	1.15 (0.74, 1.75), pd = 0.71	1.13 (0.76, 1.63), pd = 0.71	1.4 (1.18, 1.67), pd = 0.99	1.59 (1.01, 2.52), pd = 0.96	2.11 (1.03, 4.46), pd = 0.97
	Phylogenetic Signal λ , Median (90% Credible Interval)	0.26 (0.14, 0.39)	0.7 (0.54, 0.83)	0.59 (0.41, 0.77)	0.13 (0.06, 0.23)	0.69 (0.52, 0.83)	0.82 (0.67, 0.94)

Table 5.2 Model predictor effect estimates (posterior median odds ratio and 90% highest-density interval) on the presence of a plumage patch with achromatic or chromatic visual contrast values > 1, 2, and 3 JND. Model effects with a probability of direction (pd) value ≥ 0.90 are bolded in red for a negative effect and blue for a positive effect on plumage dichromatism. Phylogenetic signal (λ) for each model is provided as the median and 90% credible interval of the intraclass correlation coefficient among species.

Characteristic	Achromatic & Chromatic JND > 1, N = 77 ¹	Achromatic & Chromatic JND > 2, N = 77 ¹	Achromatic & Chromatic JND > 3, N = 77 ¹
Number of Sexually-Dimorphic Plumage Patches			
0	1 (1.3%)	34 (44%)	49 (64%)
1	4 (5.2%)	14 (18%)	10 (13%)
2	11 (14%)	7 (9.1%)	2 (2.6%)
3	10 (13%)	3 (3.9%)	2 (2.6%)
4	10 (13%)	1 (1.3%)	7 (9.1%)
5	12 (16%)	4 (5.2%)	0 (0%)
6	8 (10%)	3 (3.9%)	0 (0%)
7	4 (5.2%)	4 (5.2%)	3 (3.9%)
8	5 (6.5%)	1 (1.3%)	1 (1.3%)
9	5 (6.5%)	1 (1.3%)	1 (1.3%)
10	7 (9.1%)	5 (6.5%)	2 (2.6%)
¹ Statistics presented: n (%)			

Table 5.3 Number of sexually-dimorphic plumage patches for combined achromatic and chromatic just noticeable difference (JND) thresholds by number of *Turdus* thrush species (% of species).

Characteristic	Achromatic > 1 JND, N = 77 ¹	Achromatic > 2 JND, N = 77 ¹	Achromatic > 3 JND, N = 77 ¹	Chromatic > 1 JND, N = 77 ¹	Chromatic > 2 JND, N = 77 ¹	Chromatic > 3 JND, N = 77 ¹
Number of Sexually-Dimorphic Plumage Patches						
0	8 (10%)	41 (53%)	51 (66%)	6 (7.8%)	47 (61%)	61 (79%)
1	19 (25%)	10 (13%)	10 (13%)	15 (19%)	11 (14%)	5 (6.5%)
2	14 (18%)	9 (12%)	4 (5.2%)	22 (29%)	5 (6.5%)	3 (3.9%)
3	11 (14%)	5 (6.5%)	7 (9.1%)	11 (14%)	7 (9.1%)	2 (2.6%)
4	11 (14%)	5 (6.5%)	3 (3.9%)	14 (18%)	1 (1.3%)	2 (2.6%)
5	14 (18%)	7 (9.1%)	2 (2.6%)	9 (12%)	6 (7.8%)	4 (5.2%)

¹ Statistics presented: n (%)

Table 5.4 Number of sexually-dimorphic plumage patches for separate achromatic and chromatic just noticeable difference(JND) thresholds by number of *Turdus* thrush species (% of species).

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CHAPTER 6: SUMMARY

Visual ecology is the study of how animals use visual systems to acquire resources crucial to survival and reproduction (Cronin et al. 2014). Birds are an exemplary taxon for addressing questions relevant to visual ecology; vision is their prevailing sensory modality, and they have excellent color vision (Martin 2017). The studies of this dissertation demonstrate that 1) obligate avian brood parasitism host egg rejection behavior is a window into understanding avian vision and visual cognition under ecologically relevant circumstances (Ch. 1-4), and 2) mate recognition can be a prominent driver of plumage color evolution in birds (Ch. 5).

Avian Vision and Brood Parasitism

Vision is a key sensory modality for birds with clear fitness benefits; birds use their vision to find food, choose mates, recognize offspring, and avoid predators (Martin 2017). Avian brood parasitism is a particularly useful paradigm for addressing the question of how birds perceive color, process information about color, and make fitness-relevant decisions in response to colorful stimuli (Stoddard and Hauber 2017). Obligate interspecific avian brood parasites relinquish parental care of their own offspring and lay their eggs into the nests of unrelated host species (Davies 2000). Brood parasite host species face potential loss of reproductive fitness if they accept brood parasitic eggs laid in their nests and raise the unrelated brood parasites to fledging

(Davies 2000; Hauber 2003). After a brood parasite lays its egg(s) into the host nest, the host species can avoid fitness costs of raising brood parasites by either rejecting and removing brood parasite eggs from the nest, abandoning the nest and re-nesting elsewhere, withholding parental care to brood parasite hatchlings, or removing brood parasite hatchlings from the nest (Payne 1977). Host egg rejection experiments are exceptionally ideal for testing predictions about avian vision under semi-natural contexts because fitness benefits to egg rejection are well established (Hauber 2003; Langmore et al. 2005; Croston and Hauber 2015), artificial foreign eggs are an easily manipulated standardized static visual stimulus (Hauber et al. 2015; Igic et al. 2015), host egg rejection responses can be readily observed in the field and are highly consistent within individuals (Croston and Hauber 2014; Luro and Hauber 2017), and egg rejection experiments in general are minimally invasive with low impacts on birds' reproductive success (Hanley et al. 2015).

In Chapter 1, I present the argument that much can be learned about categorical visual perception and color discrimination in birds from avian brood parasitism egg rejection experiments, where researchers place artificial foreign eggs of different colors, shapes, and sizes into the nests of avian brood parasite host species and record their rejection/acceptance responses to the experimental eggs. Boundaries between “own egg” and “foreign egg” categories can be readily measured and assessed by designing

artificial eggs that vary continuously for a feature of interest, recording host responses to the eggs, and using statistical models to determine egg feature thresholds past which hosts will recognize eggs as foreign and reject them from the nest.

A particularly elegant experiment performed by Hanley et al. (2017) used a range of artificial eggs painted with a gradient of natural egg colors from blue-white-brown and a gradient of unnatural purple-green egg colors, and determined that birds only respond predictably to natural blue-white-brown egg colors, mainly by rejecting “browner” eggs. In Chapter 2, we placed a range of artificial eggs varying discretely in color, spotting, and size, and found that American robins (*Turdus migratorius*) reject artificial eggs in a manner consistent with a specific intolerance towards eggs resembling those of the brown-headed cowbird (*Molothrus ater*), the natural (and only) brood parasite of robins. Results from Hanley et al. (2017) and Chapter 2 strongly suggest that ecological context is crucial when trying to understand the presence, features, and boundaries of categorical perception in non-human animals, a central argument outlined in Chapter 1.

Chapters 2 and 3 provide experimental tests of own vs. foreign egg categorical boundaries using American robins (*Turdus migratorius*) as a model avian brood parasite host species. American robins are a “rejecter” host species of brown-headed cowbirds (*Molothrus ater*) because they consistently and predictably reject cowbird eggs

(Rothstein 1982; Croston and Hauber 2014). Robins' robust egg rejection responses allow for rigorous experimental tests of the interplay between egg features and avian visual perception in determining foreign egg rejection/acceptance decisions (i.e., categorizing an experimental egg as "own" or "foreign" and accepting/rejecting the egg accordingly). Chapter 2 establishes a baseline for understanding which egg features American robins cue in on when assessing eggs in their nests. We found robins responded most strongly and consistently to egg background coloration that diverges from the natural robin egg blue-green coloration toward a brown color resembling the background color of natural cowbird eggs. Robins also responded to differences in egg size and spotting, but to a lesser degree than to changes in background coloration. Our results support a "cowbird egg intolerance" hypothesis for robin egg rejection, where robins focus mainly on egg features resembling those of brown-headed cowbird eggs and will categorize and reject a foreign egg when it appears similar to a cowbird egg.

For Chapter 3, we address a previously untested question of how robins respond simultaneously to both chromatic and spatial features of eggs. We created a range of unique checkerboard-patterned eggs consisting of blue and yellow squares of different sizes. The checkerboard patterns were carefully designed to mimic the color of natural blue-green robin eggs when the squares cannot be spatially resolved as separate blue and yellow squares and visually blend into a uniform blue-green color. We placed the

checkerboard-patterned eggs into robin nests, recorded their rejection responses to the various checkerboard square sizes, and determined that, surprisingly, robins will accept some model eggs with novel blue-yellow checkerboard patterns that appear to be easily distinguishable from natural robin eggs from the human point of view. Our results provide a concrete example of the inappropriateness of assuming our own visual experience is similar to the visual experience of birds (and other non-human animals) (Caves et al. 2019). Chapter 3 results also demonstrate the possibility that brood parasites could exploit limitations of host species visual acuity and lay eggs that appear similar to host eggs from the host species point of view due to visual blending of the brood parasite egg spotting and background color.

Chapter 4 indirectly addresses two important questions about avian vision and avian brood parasite host egg rejection: how and why do differences in bird species visual phenotypes evolve, and do these differences in vision translate into differences in foreign egg recognition and egg rejection behavior? Differences in bird species visual field range, visual acuity, and color perception seem to be related to differences in foraging ecology between species (Martin 2017). In Chapter 4, we use a comparative phylogenetic analysis to test the idea that differences in phenotypes related to foraging strategies and diets correlate with differences in foreign egg recognition and rejection behavior. We found that species with omnivorous, frugivorous, and animal-centric

diets reject foreign eggs more frequently than species with highly granivorous diets. We also found that species possessing large body sizes and that forage arboreally rather than aerially or on the ground are much better at rejecting foreign eggs. We suggest that differences in species foraging ecology that explain variation in egg rejection behavior likely also reflect differences in species visual perception, including visual acuity and color perception. Therefore, avian species phenotypes which evolved to exploit food resources could directly constrain and affect species later ability to recognize and respond to foreign eggs laid in their nests.

Avian Plumage Color

Birds are arguably the most colorful taxonomic group of terrestrial animals. Avian plumage colors are extremely diverse and likely contain a variety of messages about an individual, including species, sex, age, breeding status, dominance status, and identity (Hill and McGraw 2006). Chapter 5 addresses the question of why sexual dichromatism, distinct differences in plumage color between males and females, is present in some but not all species. I collected plumage spectral reflectance measurements from male and female museum bird skins for 77 species of true thrush (*Turdus* spp.). I ran these plumage spectral reflectance measurements through a model of European blackbird (*T. merula*) vision to establish which true thrush species are sexually-dichromatic and which are sexually-monochromatic. We then tested the

hypothesis that if plumage sexual dichromatism is predominantly used to recognize a compatible mate of the same species, then ecological pressures favoring fast and accurate species and mate recognition should be positively correlated with plumage sexual dichromatism. We identified and predicted that pressures likely to favor rapid and accurate species recognition, and thus, plumage sexual dichromatism, include reduced time to breed (i.e., migration to and from breeding grounds rather than no migration, and shorter breeding season length), greater difficulty in identifying a compatible breeding mate of the same species (i.e., high breeding sympatry with many heterospecific true thrush species), and greater effort necessary to locate a compatible breeding mate (i.e., larger breeding range sizes and mainland rather than island residency). We used comparative phylogenetic models and found that plumage sexual dichromatism patterns among true thrush species were consistent with our predictions that need for fast and accurate species and breeding mate recognition increases the likelihood that a species will have plumage sexual dichromatism. Our results provide correlational evidence in favor of recognition as a main driver of differences in appearance between sexes.

Conclusions

In summary, the results of this dissertation demonstrate the importance and utility of linking the perception and the function of conspicuous traits to fitness-relevant

contexts to the taxon of interest. Recognition, including both recognition of own offspring and suitable breeding mates, is a powerful lens through which we can view and test the perceptual and cognitive capabilities of animals. Many fitness effects of avian brood parasitism on host species have been extensively studied, documented, and supported. I encourage future work to further capitalize on the strong foundations of avian brood parasitism research and continue to reveal insights into avian visual perception and cognition using egg rejection experiments as a model system.

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