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Testing assumptions of coevolution in an egg-rejecting brood parasite host: Uncovering sensory, cognitive, and evolutionary drivers of responses to parasitism in American robins (Turdus migratorius)

Rebecca Croston
Graduate Center, City University of New York

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Testing assumptions of coevolution in an egg-rejecting brood parasite host: Uncovering sensory, cognitive, and evolutionary drivers of responses to parasitism in American robins (Turdus migratorius)

Rebecca Croston

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This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Date

Chair of Examination Committee
Dr. Mark E. Hauber
Hunter College, CUNY

Date

Executive Officer
Dr. Laurel A. Eckhardt

Dr. Jennifer A. Basil
Brooklyn College, CUNY

Dr. David C. Lahti
Queens College, CUNY

Dr. Lisa L. Manne
College of Staten Island, CUNY

Dr. Michael S. Webster
Cornell University, Lab of Ornithology
Abstract

Testing assumptions of coevolution in an egg-rejecting brood parasite host: Uncovering sensory, cognitive, and evolutionary drivers of responses to parasitism in American robins (Turdus migratorius)

By Rebecca Croston

Advisor: Dr. Mark E. Hauber

Hosts of brood parasitic birds face fitness costs associated with rearing unrelated offspring. In response, the recognition and rejection of parasitic eggs is a common host defense. Brown-headed cowbirds (Molothrus ater) challenge coevolutionary theory, because although they exploit over 200 host species, they lay non-mimetic eggs, and most hosts do not combat cowbird parasitism with egg rejection. American robins (Turdus migratorius) are one of a handful of cowbird hosts known to recognize and remove cowbird eggs from the nest. I addressed the mechanistic and evolutionary drivers of egg rejection in this host species, by disentangling the roles of spectral tuning and visual physiology on the behavioral outcome of egg rejection, by estimating the costs of parasitism which may drive egg rejection behavior, and by addressing the reciprocal effects parasitism on host egg color variation and its role in mediating rejection decisions. I also test assumptions underlying the evolvability of host egg rejection responses in this system. In Chapter 1, I lay out an overview of brood parasitism as a reproductive strategy and brood parasite-host ecology, and highlight evolutionary mechanisms and consequences of coevolution in these systems. In Chapter 2, I test the hypothesis that foreign egg rejection is driven proximately by perceivable differences in ground color between
host and parasitic eggs across the entire avian spectral sensitivity range. I show that the rejection of artificially dyed eggs is mediated by input from all four avian single-cone photoreceptors, and that more divergent model ‘parasitic’ eggs are indeed rejected at higher rates. However, the cowbird egg does not conform to this prediction, because both model and real cowbird eggs are rejected in 100% of experimental trials despite their lower overall discriminability from robin eggs. This may indicate a cowbird-egg specific rejection response in robins. In Chapter 3, I test a critical assumption underlying the evolution of cowbird-specific egg rejection responses in robins, by assessing the hypothesis that cowbird parasitism imposes recoverable costs on robin hosts. My results indicate that cowbird chicks fare poorly when reared alongside robin chicks, but parasitism per se still reduces nesting success for robins; thus, rejection of cowbird eggs serves a function to eliminate the cost of parasitism. In Chapter 4, I examine a critical assumption underlying all of host-parasite coevolutionary theory, namely that host defenses can evolve genetically in response to parasitism. I address the hypothesis that egg rejection is repeatable in our study population, as repeatability is prerequisite to the evolution and spread of a behavioral trait, including a predictor of the trait’s genetic heritability. As predicted, egg rejection behavior in American robins was found to be highly repeatable for intermediately-rejected model egg colors within the same nesting attempt, irrespective of potentially confounding ecological and temporal factors. Finally, in Chapter 5, I test predictions stemming from alternate hypotheses that egg rejection evolved in response to cowbird (non-mimetic) versus conspecific (mimetic) parasitism, by investigating the degree of color variation within robins’
own clutches, and the effect of experimentally manipulating intraclutch color variation. I used both observational and experimental data, and found that egg color varies more between clutches than among egg within a single clutch, yet experimental manipulated intraclutch color variation did not affect rejection rates. These results support the scenario of historical parasitism by non-mimetic parasites. Variation among the findings of similar studies pertaining to hosts of mimetic parasites may be explained by hosts' use of different cognitive mechanisms in the decision to reject foreign eggs. However, for hosts of non-mimetic parasites, investigating egg color variation and its effect on egg rejection is not informative about different cognitive decision-making rules, as predictions under each mechanism are similar - that there will be no effect of a history of parasitism on intraclutch color variation (observational patterns) or rejection rate (experimental data). This body of research presents compelling evidence in support of egg rejection by robins as a specific response to historical cowbird parasitism, and has highlighted important components of the sensory, cognitive, functional and evolutionary processes underlying egg rejection in this paradoxical brood parasite-host system.
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For his many years of skillful guidance in each step of the arduous research process, I sincerely thank Mark Hauber. Through his leadership and mentorship, I have learned not only about the many paradoxes remaining in the relationships between brood parasites and their hosts, but also how to navigate the complex, fascinating, and often daunting process of scientific inquiry. His continual support has been the single biggest influence on my research, and on my growth as a researcher, and I’m truly grateful to have had the opportunity to learn from him throughout these last five years, and into the future.

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CHAPTER 1

The Ecology of Avian Brood Parasitism

Croston, Rebecca¹* & Hauber, Mark E.¹,²

1. Doctoral Program in Biology, Subprogram in Ecology, Evolutionary Biology and Behavior, The Graduate Center, City University of New York, New York 10016, USA.

2. Department of Psychology, Hunter College and Doctoral Program in Psychology, The Graduate Center, City University of New York, New York 10065, USA.

*Corresponding author. Mailing address: c/o Hauber Lab, Department of Psychology, Hunter College, 695 Park Avenue, New York, New York, 10065, USA. Telephone: +1/212 396 6445, e-mail: RCroston@gc.cuny.edu

Brood parasitism as a reproductive strategy

Avian brood parasitism, or the laying of one’s eggs in the nest of another individual, is a reproductive strategy whereby parasites foist the cost of rearing their offspring onto another individual, the host (Davies, 2000). Brood parasitism may be facultative at the species or individual levels, with some eggs incubated by the mother and others laid in foreign nests, or obligate. Brood parasitism may also be intraspecific, with eggs laid in other nests of the parasite’s own species, or interspecific, with all eggs laid in the nests of other species. Cowbirds and cuckoos are the most commonly studied avian brood parasites (Davies, 2000), although obligate interspecific brood parasitism has evolved at least 7 separate times among various avian clades, including cowbirds (Icteridae), honeyguides (Indicatoridae), Old World cuckoos (Cuculinae), twice in the New World cuckoos (Neomorphinae), indigobirds and their allies (Ploceidae), and the Black-headed duck (Anatidae).

For the parasite, benefits include increased fecundity due to greater allocation of resources toward mating and producing more eggs rather than defending nests, incubating eggs, and feeding young. For hosts of brood parasitic birds, the costs of parasitism range from lower nestling growth rate, due to competition with larger and more competitive parasitic offspring (cowbirds, whydahs), to total loss of breeding by the abandonment of parasitized broods (cowbirds, cuckoos), the eviction of all host eggs by the early-hatching parasites (cuckoos), or the killing of host hatchlings by parasitic hatchlings (cuckoos, honeyguides) (Kilner, 2005; Servedio & Hauber, 2006). These costs exert reciprocal natural selection on parasites and hosts, such that in many cases host-parasite interactions
result in escalating coevolution between intimately tied and interdependent species (Langmore et al., 2003). In turn, many hosts are able to discriminate against and reject foreign eggs or chicks based on visual, acoustic, or multimodal sensory cues (Cassey et al., 2008). The eggs of many brood parasites, for example, mimic those of their hosts (to deceive hosts to accept), have harder shells (to impede rejection by puncture), and require slightly shorter incubation times (causing a size advantage for parasitic nestlings) (Davies, 2000).

**Evolution and Maintenance**

There are two major hypotheses that have been implemented in attempting to explain the evolution and maintenance of the complex and often paradoxical reproductive strategies that fall under brood parasitism (Rothstein & Robinson, 1998). The evolutionary lag hypothesis posits that rejection is almost always more adaptive than acceptance, and 1) hosts should accept parasitism only because they have not yet evolved mechanisms for defense against parasites, and/or 2) parasites fail with certain hosts because they have not yet evolved mechanisms for overcoming existing host defenses. Historically recent contact, due to natural or anthropogenic change (e.g., deforestation, forest fragmentation), the acceptance of foreign eggs even when these do not resemble host eggs (nonmimetic), and a high cost of parasitism without apparent defenses to prevent and recognize parasitism, all suggest that evolutionary lag is the mechanism for host acceptance of parasitic eggs, but as it is difficult to test this hypothesis directly, it is often the default or fall-back explanation (Peer & Sealy, 2004).
In contrast, the evolutionary equilibrium hypothesis posits that hosts accept parasitism only because the cost associated with the rejection of parasitic eggs is greater than the cost of rearing cowbird offspring (Klippenstine & Sealy, 2008). Rejection costs may be incurred via misrecognition of parasitic eggs, so that hosts mistakenly eject their own eggs, or via rejection costs, when hosts accidentally damage their own eggs while attempting to remove parasitic eggs. Host-egg mimicry and increased eggshell thickness by parasitic eggs have both evolved repeatedly in diverse lineages of obligate parasites, rendering the recognition and removal of parasitic eggs more costly, and thereby increasing selective pressure to accept parasitism or delay the evolution of costly and error-prone discrimination mechanisms to reject parasites.

The Coevolutionary ‘Arms-Race’

Egg Mimicry

Most host defenses against costly parasitism occur at the egg stage with the recognition and removal of parasitic eggs (Figure 1.1). This ability may have evolved from behaviors such as nest sanitation (removal of fecal sacs and broken shells), and morphological traits such as large bills, which serve as preadaptations for removal of parasitic eggs (Peer & Sealy, 2004). Egg recognition thereby exerts selective pressure on the parasites to lay eggs that mimic in appearance those of their host, and reciprocal pressure on hosts to fine-tune their discriminative abilities. This “arms race” is at the heart of brood parasitism as a coevolutionary phenomenon. The degree of egg mimicry and concurrent host specialization varies dramatically among parasitic clades. A recent study by Klippenstine
and Sealy (2008) has shown that grassland cowbird hosts possess the ability to discriminate between and reject foreign eggs when the eggs differ dramatically (in color and maculation) from their own eggs, but these same species do not eject real or model cowbird eggs. This suggests that a more generalized form of egg mimicry than that employed by cuckoos, and that Brown-headed cowbird eggs loosely mimic a wide range of potential grassland hosts. In contrast, individual females of many old world cuckoos, lay eggs that are specific to particular hosts — that is, they mimic eggs of a specific subset of their host species. Generalist cowbirds, by contrast, do not lay highly mimetic eggs, and parasitize a wide range of hosts rather than specializing on a particular species of host or host-egg race (Figure 1.1). Recognition and removal of parasitic eggs is based largely on differences between own and foreign eggs in background color, with size, shape, and maculation acting in various combinations to elicit egg rejection. A major caveat in studies of degree of egg color matching to date has been that eggs are assessed according to the human visual system. As many birds possess a fourth, UV-sensitive photoreceptor type relevant in behavioral decision-making, biologically realistic sensory models should be used in future studies to determine the parameters eliciting egg rejection (Cassey et al., 2008; Honza et al., 2007).
Figure 1-1. Common Cuckoos (*Cuculus canorus*) parasitizing Common Redstarts (*Phoenicurus phoenicurus*) in Europe lay eggs whose color mimics closely host egg colors (the parasite egg is the slightly larger egg). In contrast (inset), Brown-headed Cowbirds (*Molothrus ater*) in North America lay speckled eggs which do not resemble the pure white eggs of one of their many hosts, the Eastern Phoebe (*Sayornis phoebe*). (Courtesy of T. Grim & M. Hauber)

Nestling mimicry

If constraints surrounding egg recognition and removal make rejection at the egg stage too costly, the recognition and rejection of nestlings may provide an effective alternative defense strategy for host species, ultimately resulting in plumage, mouth, and begging call mimicry (Langmore et al., 2003; Anderson et al., 2009). For post-hatching discrimination to evolve, parasitism rates must be sufficiently high to outweigh the cost of recognition errors, and hosts must have sufficiently high fecundity to bear the cost of
mistakenly rejecting their own chicks (Langmore et al., 2003). Host rejection of parasitic nestlings has been demonstrated in Superb Fairy-wrens (*Malurus cyaneus*), and may have selected for the evolution of nestling begging call mimicry in Bronze Cuckoos (*Chrysococcyx basalis*) (Langmore et al., 2003). Nestling discrimination by hosts is, however, relatively rare, despite hosts having various behavioral and cognitive traits that would enable such behavior. This is likely because nestling discrimination can only evolve when egg discrimination has failed (Grim, 2006).

Hosts may discriminate nestlings using cues such as size, color, vocalization, and overall clutch size. Mouth coloration and gape patterns of parasitic nestlings can stimulate higher rates of provisioning by host parents by serving as a supernormal stimulus and enabling parasites to outcompete host young (Kilner et al., 1999). In parasitic indigobirds, nestling flange markings may resemble those of healthy hosts in order to stimulate greater provisioning (Hauber & Kilner, 2007) and to avoid discrimination through reduced feeding by host parents (Schuetz, 2005).

**Identity Crisis?**

If parasitic nestlings are not exposed to conspecifics during development, then how are they able to identify members of their own species with which to mate? Rather than relying solely on cues learned from parents and nestmates, brood parasites must employ some other mechanism for species recognition in order to avoid mistakenly courting heterospecifics. Brown-headed cowbirds (*Molothrus ater*) seem to rely on a combination of self-referent phenotype matching and a “password” like vocal trigger that unlocks
learning of species-specific cues at their first encounter with a conspecific (Hauber et al., 2000, 2001). Such a combination of developmental paths and recognition mechanism may apply to brood parasites more generally, and could represent a difficult-to-evolve behavioral algorithm, as was seen recently in an experimental study where male facultative interspecific brood parasitic ducks mistakenly courted females of the host species instead of their own (Sorenson et al., 2010).

**Conservation Impacts of Brood Parasitism**

As a widespread generalist brood parasite, the native brown-headed cowbird poses a conservation threat to several of its North American passerine hosts. Selective pressure resulting from cowbird parasitism is likely higher now than in the past, owing to increased suitable habitat provided by deforestation, leaving more and novel hosts vulnerable to parasitism by increasing numbers of cowbirds (Davis & Sealy, 2000). For brown-headed cowbird host populations already in decline, such as the endangered Kirtland’s Warblers (*Dendroica kirtlandii*), Black-capped Vireos (*Vireo atricapilla*), Least Bell’s Vireos (*Vireo bellii pusillus*), and Southwestern Willow Flycatchers (*Empidonax traillii extimus*), the effects of this can be devastating, and human control of cowbird population size may be necessary to prevent local extinctions (Smith et al., 2000). This is a particular consideration for conservation biologists working with hosts of generalist brood parasites, because even when a species declines in number it may continue to be parasitized at high rates, since as generalist parasites, the cowbird population will not be impacted reciprocally with that of individual host species. These applied aspects of host-parasite interactions confirm that scientifically informed
conservation management is critical for the efficient and productive planning and implementation of long term goals (Hauber, 2009; Parker et al., 2010).
References


Spectral tuning and perceptual differences do not explain the rejection of brood parasitic eggs by American robins (*Turdus migratorius*)

Croston, Rebecca* and Hauber, Mark E.¹,²

1. Doctoral Program in Biology, Subprogram in Ecology, Evolutionary Biology and Behavior, The Graduate Center, City University of New York, New York 10016, USA.

2. Department of Psychology, Hunter College and Doctoral Program in Psychology, The Graduate Center, City University of New York, New York 10065, USA.

*Corresponding author. Mailing address: c/o Hauber Lab, Department of Psychology, Hunter College, 695 Park Avenue, New York, New York, 10065, USA. Telephone: +1/212 396 6445, e-mail: RCroston@gc.cuny.edu
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avian visual modeling, brood parasitism, brown-headed cowbird, egg rejection, ultraviolet reflectance, visual ecology
Abstract

By laying their eggs in the nests of other birds, avian brood parasites impose the cost of rearing young upon their hosts. The recognition and rejection of foreign eggs are primary host defenses against costly brood parasitism. Hosts of parasitic brown-headed cowbirds (*Molothrus ater*) challenge coevolutionary theory because most cowbird hosts accept parasitic eggs despite their drastically different appearance from the hosts’ own eggs. American robins (*Turdus migratorius*) are one of only 10% of the over 200 potential cowbird host species to robustly reject parasitic eggs, but the mechanisms driving the sensory bases of foreign egg rejection remain elusive. Our research combined avian visual perceptual modeling and behavioral experimentation to investigate chromatic cues eliciting parasitic egg rejection in American robins. We assessed the effects of perceivable background color differences between real host and model parasite eggs, across all four avian photoreceptors, on rates of rejection of model eggs spanning in color across the entire avian spectral sensitivity range, and including immaculate model eggs matching the natural color of robin or cowbird eggs. The results suggest that egg rejection in robins is driven by the overall perceivable difference in color between own and artificial eggs, and input from all four single-cone avian photoreceptors affects the rejection decision. The results, however, also reveal that when viewed by the avian eye, natural cowbird eggs appear more similar in background color to robin eggs than predicted by the high rejection rate of these parasitic eggs. This suggests that robins respond specifically to parasitism by cowbirds, despite an apparent lack of sensory tuning toward the detection of the background color of cowbird eggs.
Introduction

Approximately 1% of all avian species are obligate brood parasites (Payne 1977), which lay their eggs in the nest of other bird species (the hosts), thereby releasing themselves from the temporal and energetic costs of rearing their own offspring (Davies 2000). Hosts of brood parasites experience depressed reproductive success (Røskaft et al. 1990; Payne and Payne 1997; Øien et al. 1998; Lorenzana and Sealy 2001; Hauber 2002, 2003). The recognition and removal of parasitic eggs is a primary and effective host defense against brood parasitism (Rothstein 1975; Rothstein and Robinson 1998; Kilner and Langmore 2011; Grim et al. 2011), which in turn exerts selective pressure on the parasites to lay eggs that mimic host eggs in appearance (Davies and Brooke 1989; Moksnes and Røskaft 1995; Stoddard and Stevens 2011). The result is a reciprocal evolutionary pressure on hosts to fine-tune their sensory, discriminatory, and rejection abilities in response to increasingly more mimetic parasites (Anderson et al. 2009).

The degree of host-parasite egg mimicry and concurrent host specialization varies dramatically among parasitic lineages and their hosts (Rothstein 1990). Brown-headed cowbirds (*Molothrus ater*; hereafter, cowbirds) are generalist obligate brood parasites, and lay eggs in the nests of 245 North American passerine species (Friedmann 1929, 1971, Lowther 2012). Cowbird nestlings, while less virulent than those of many other obligate brood parasite lineages (Hauber 2003a; Kilner 2003; Kilner et al. 2004), are typically larger and more competitive than their hosts’ nestlings, and able to monopolize hosts’ parental care leading to reduced growth of nest mates, especially in small-bodied host species (Payne 1977; Rothstein 1990; Slagsvold 1998; Kilpatrick 2002; Hauber
Unlike the historically well-studied common cuckoos (*Cuculus canorus*; Davies and Brooke 1988, 1989; Mokneses and Røskaft 1995; Aviles 2008; Stoddard and Stevens 2010, 2011; Iggic et al. 2012; Stoddard and Kilner 2013), cowbird eggs do not appear to closely mimic those of their hosts, when assessed by human vision (Friedmann 1929; Rothstein 1982; Klippenstine and Sealy 2010). Given the many costs of providing care for unrelated offspring, the prevalence of egg rejection among hosts of egg-mimetic brood parasites (Davies 2000), and the wide range of cowbird hosts with highly variable egg appearances, it is paradoxical that few cowbird host species eject the parasite’s eggs (Hosoi and Rothstein 2000).

The removal of parasitic eggs and other foreign objects may be driven mechanistically by differences in shape (Moskat et al. 2003a; Guigueno and Sealy 2012), size (Marchetti 2000), maculation (Lahti and Lahti 2002; Lopez-de-Hierro and Moreno-Rueda 2010; Moskat et al. 2010), ultraviolet (UV) reflectance (Honza et al. 2007; Honza and Polacikova 2008), overall brightness (Lahti 2006), color difference in a particular part of the egg shell (e.g. blunt pole; Polacikova et al. 2010), or inherent aspects of background coloration (Moskat et al. 2008; Ban et al. 2013). Hosts may also respond to overall differences by integrating several different visual and tactile characteristics (Rothstein 1982; Spottiswoode and Stevens, 2010; de la Colina et al. 2012). In many earlier studies of brood parasitic egg rejection, the artificial egg stimulus and resulting analyses relied on either human assessment of egg colors or comparison based on spectrophotometric reflectance measures of host and parasite eggs (Croston and Hauber 2010). Because such analyses do not fully or specifically account for differences between
human and avian vision, including avian UV-sensitivity (Cuthill et al. 2000), it is necessary to use UV-inclusive reflectance spectrophotometric data (Cherry and Bennet 2001; Honza et al. 2007; Cherry et al. 2007a,b) in conjunction with the known spectral sensitivities of focal host bird species (Hart et al. 2000) in order to establish the perceptual thresholds of own-foreign egg color discrimination (e.g. Aviles 2008; Cassey et al. 2008; Langmore et al. 2009; Igic et al. 2010, 2012; Stoddard and Stevens 2010). In doing so, we can test the role of avian-perceived color differences in eliciting egg rejection decisions.

American robins (*Turdus migratorius*; hereafter, robins) are one of only ca. 26 cowbird host species which grasp and eject cowbird eggs (Peer and Sealy 2004) in ca. 100% of trials, where nests are experimentally parasitized with real or model parasite eggs, in areas of sympatry with cowbirds (Rothstein 1975; Briskie et al. 1992). To the human observer, cowbird and robin eggs differ markedly in both background coloration (i.e. external ground color of the eggshell) and maculation (Friedmann 1929; Fig. 2.1). Physical reflectance spectra also show differences in background coloration in both the UV and human-visible parts of the light spectrum (Underwood and Sealy 2008; Fig. 2.1). Previous work only assessed discontinuous, two-character state differences between cowbird and robin eggs in size (robin-sized vs. cowbird-sized), background color (robin-colored vs. cowbird colored), maculation (presence vs. absence; Friedmann 1929; Rothstein 1982) and shape (host vs. cowbird shaped; egg vs. non-egg shaped; Underwood and Sealy 2006), and neither quantified physical color traits nor modeled avian perception of the appearance of natural or experimental eggs. Therefore, it remains
unexplored whether robins’ cues for identifying and rejecting foreign cowbird eggs lie in finer-scale continuous differences in background color perception between own and foreign eggs, across any part of the avian-visible light spectrum.

Here, we focus on the role of egg background coloration in eliciting egg ejection in robins by manipulating it semi-continuously using artificial eggs ranging in color across the avian-visible light-reflectance spectrum. We first evaluate the hypothesis that (i) similarity between own and foreign egg background color is an important cue for egg rejection (Davies and Brooke 1988, 1989; Moskat et al. 2003a). To this aim, we test the prediction that eggs of different colors, ranging across the entire spectral sensitivity range of songbirds (Aidala et al. 2012), will be rejected at predictably different rates such that artificial eggs with colors more perceivably different from the hosts’ own egg colors will be more likely to be rejected (Cassey et al. 2008; Aviles et al. 2010; Stoddard and Stevens 2010). We then investigate the alternative hypotheses that (ii) robins specifically reject cowbird-egg colored foreign eggs, or (iii) robins specifically reject foreign eggs based on perceivable color differences in the UV part of the avian visible spectrum, as this has received particular attention in brood parasite literature (e.g. UV-matching hypothesis, Cherry and Bennett 2001), and specific response to differences in UV reflectance has been demonstrated in congeners of the American robin, the European blackbird *T. merula* and song thrush *T. philomelos* (Honza et al. 2007). We set out to model effects of avian-perceived differences in color on rejection rates of model parasitic eggs following prior perceptual modeling work on foreign egg ejection in the song thrush (Cassey et al. 2008). We evaluate the effects of differences in egg color across the
sensitivity ranges of each photoreceptor, including areas of overlapping sensitivities, by using avian visual modeling to evaluate effects of relative photoreceptor catches and their interactions on rejection rates across model parasitic eggs (hypothesis i). This allows us to assess which of the four avian photoreceptors, including the UV-sensitive cone (hypothesis iii), contribute consistently to the decision to reject foreign eggs. Finally, we investigate the predictive value of the relationship between overall perceivable color differences and experimentally elicited egg rejection in our experiment on the perceivable difference in background color and the published likelihood of egg rejection by robins in response to natural conspecific and parasite eggs (hypothesis ii).

Methods

Study site and nests

In May-July 2010 and 2011, we monitored nesting activities of American robins in and around Ithaca, Tompkins County, NY, USA. Cowbird eggs are rarely found in robin nests, and during the course of our study (N = 64 nesting attempts included in this study), we only detected a single cowbird egg laid in an abandoned robin nest (Fig. 2.1). Nests were located by searching in and around natural and human-made structures, as robins show high nesting densities near human settlements (Sallabanks and James 1999; RC pers. obs.). Additional nests were located with the help of local residents recruited using various signboards, local internet list-serves, and internet advertisements (following Hauber 2003b; Wagner et al. 2013).
Fig. 2.1 Representative reflectance spectra of natural and model American robin and brown-headed cowbird egg background colors. Inset shows a natural cowbird egg (beige, spotted) found in a robin nest with a natural robin egg, from Ithaca (NY), USA.

Upon finding a nest containing two or more host eggs, we numbered all existing eggs with a non-toxic felt-tip pen (Sharpie™ brand, black), and artificially parasitized the nest by adding one plaster-of-Paris cowbird-sized egg (see below for details on artificial eggs) to the clutch. Nests were parasitized throughout both laying and incubation as available, and timing of parasitism (day in the nesting cycle) was included as a potential predictor of response to parasitism in our analysis (see Data Analysis, below). We removed no host eggs during this experiment, following prior work on this cowbird host (Briskie et al. 1992), and instead mirrored cases of natural parasitism without egg-removal by the female cowbird (reviewed in Sealy 1992). In other, European Turdus species (Moskat et al. 2003b; Honza et al. 2005, 2007), egg rejection rates were not dependent on replacing or adding artificial eggs (Grim et al. 2011). We monitored the
nest by returning daily, checking on previously marked eggs, marking any additional eggs, and determining the status of the artificial egg.

As robins reject model cowbird-sized eggs by grasping and ejecting these from the nest (Rothstein 1975), eggs were considered ejected if they were not present in the nest the following day, except when hatching or full predation (indicated by the absence of all eggs from the nest) occurred. No nests were abandoned as a result of experimental manipulation. Eggs were considered accepted if they remained in the nest for 6 consecutive days, after which the artificial egg was removed (following Grim et al. 2011 for other Turdus species). Each nest was parasitized multiple times in order to test for possible effects of presentation order on rejection rate; a single model egg was added whenever one had previously been ejected or accepted. Prior to any statistical tests, the data were randomized to avoid pseudoreplication, such that only one presentation per nest is included in the analysis, and each nest is included only once. We also avoided including data replicated within nesting pairs and across two consecutive nesting attempts by conservatively assuming that nest ownership was shared between any two nests located within ~10 m of each other throughout the season. For each nest, we recorded the site location, parasitic model egg color, timing of artificial parasitism (day in the incubation cycle), presentation number, and clutch size.

All nests were monitored until hatching in order to assess timing of the onset of each treatment relative to the laying and incubation cycle (typical robin incubation period: 12 - 14 days; Sallabanks and James 1999). This study was conducted on private
properties with the express consent of the landowners, and followed the protocol approved by the Institutional Animal Care and Use Committed of Hunter College (# MH 2/13-T3).

Artificial eggs

Artificial eggs were molded from plaster-of-Paris, following the dimensions of brown-headed cowbird eggs. All eggs weighed between 2.6 - 3.4 g, and measured 21 mm x 16 mm, based on the documented average dimensions (21.4 x 16.4 mm, 3.03 g; Lowther 1993) of cowbird eggs near Ithaca. The eggs were then painted with either non-toxic acrylic or latex house paint (Behr PREMIUM PLUS™ Interior Paint), using colors with reflectance peaking at wavelength intervals spanning the avian visual sensitivity range. Colors were chosen by inspecting the characteristic shape of their reflectance spectra, as determined using avian-visible range spectrophotometric measurements (described below; Fig. 2.2), based on both the wavelengths of peak quantum receptor catches, as ‘color’ is determined by the relative rather than absolute receptor catches (Endler and Mielke 2005), and the wavelength at peak reflectance, which influences hue (Endler 1990). Red, yellow, and blue model egg colors peak at even intervals across the avian visual range (wavelength 650nm, 550nm, and 450nm respectively). Additional experimental eggs were dyed to resemble (mimic) the background color of otherwise maculated, real (natural) cowbird eggs (“BHCO ground”), or the ‘background’ color of immaculate, real (natural) robin eggs (“AMRO ground”; Fig. 2.1, see below for measurement methods). As the importance of ultraviolet reflectance has also been demonstrated for some avian host-parasite systems to establish mimicry and to mediate
hosts’ egg rejection responses to some brood parasitic species’ eggs (Cherry and Bennett 2001; Honza et al. 2007), we also included “UV-blocked” model eggs. These were painted the same color as AMRO ground model eggs, and then coated with unscented SPF 50 lotion sunscreen to cut out reflectance specifically in the ultraviolet part of the avian visual spectrum (sensu Aviles et al. 2005; Honza and Polacikova 2008). While all model eggs differ slightly in texture from real, unmanipulated robin eggs, the presence of dried sunblock lotion did not affect the texture of the model egg surface (RC pers. observ.). That our uncoated mimetic model eggs were never rejected, and only one UV-blocked egg was ever rejected, suggests that any unquantified difference in texture between real and model eggs also did not affect rates of model egg rejection.

Spectral measurements

We characterized model and natural egg color across the entire avian visual spectrum by measuring spectral reflectance using a high resolution spectrometer with deuterium tungsten halogen light source and 455μm solarization-resistant shielded cable (Ocean Optics Jaz spectrometer with UV-VIS light source, Ocean Optics Inc., Dunedin, FL, USA). Measurements were taken using a fiber optic probe held perpendicular to the egg surface for each individual measurement. The spectrometer was calibrated and spectra expressed relative to a Spectralon reflectance standard (WS-1, Ocean Optics, Inc.), which reflects > 95% of UV and visible light, and a fully dark standard (a paper-box with black-felt entry hole to block all light from entering; Igic et al. 2010), to account for baseline noise in the spectrophotometer. The percent reflectance at each wavelength
was calculated automatically with reference to the light and dark standards. To minimize measurement error, the spectrometer was calibrated repeatedly throughout sampling.

![Graph showing observed rejection rates for different egg types](image)

**Fig. 2.2** Summaries of means and 95% Wilson confidence intervals for rejection rates in response to each model egg type. Colors listed beneath columns denote model egg type. A representative reflectance spectrum and a photograph of each model egg type are shown below.

Nine measurements were taken for each model egg, three measurements each at the blunt pole, middle, and sharp pole. These measurements were averaged for each model and natural egg, yielding the average spectral reflectance curve for each. For natural robin eggs, spectral measures were taken on the day after clutch completion. Representative spectra for natural and model eggs are shown in Figs 2.1, 2.2.
Data analysis

Describing model egg color

First, we quantified color variation between natural robin eggs and each model egg color for each photoreceptor. We divided the spectral sensitivity range into four regions based on the maximal sensitivities of each of the four photoreceptors of the congeneric European blackbird *T. merula* as described in Hart et al. 2000. Values of reflectance ratios \( R_{300-400/300-700}, R_{400-475/300-700}, R_{475-550/300-700}, R_{550-700} \) were used as estimates of UVS (ultra-violet sensitive) photon catch, SWS (short wavelength sensitive) photon catch, MWS (medium wavelength sensitive) photon catch, and LWS (long wavelength sensitive) photon catch respectively (Sheldon et al. 1999). Quantitative descriptions of all model egg colors are listed in Table 2.1 with photos and reflectance spectra shown in Figs 2.1, 2.2.

Comparing egg rejection responses between model eggs

Prior to any analysis and in order to avoid pseudoreplication, data were randomized such that only one presentation at each nest was included in the analyses, and each nest was included in an analysis only once. To test for independence of frequency of rejection (i.e. ejection) among different colored model eggs, we conducted a \( \chi^2 \) test for a multi-way contingency table of acceptance and rejection [egg color X outcome (accept, reject)]. When expected cell counts were less than 5, we analyzed differences among ranked rejection rates across colors using Kruskal-Wallis tests. Post hoc t-tests were then used to evaluate differences among rejection rates for specific color pairs. As each nest was artificially parasitized multiple times, we applied additional Kruskal-Wallis tests to
Table 2.1

Table 2.1a Mean ± standard deviation of differences in photon catch (as percent reflectance [%]) across photoreceptors and in response to different model egg types, UVS, SWS, MWS, and LWS refer to single-cone photoreceptor types ultraviolet-sensitive, short-wavelength sensitive, medium-wavelength sensitive, and long-wavelength sensitive, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Yellow</th>
<th>Red</th>
<th>Blue</th>
<th>AMRO ground</th>
<th>UV-blocked</th>
<th>BHCO ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVS</td>
<td>7.301 ± 1.03</td>
<td>6.919 ± 1.16</td>
<td>3.741 ± 2.66</td>
<td>0.426 ± 1.26</td>
<td>5.710 ± 2.05</td>
<td>0.733 ± 2.21</td>
</tr>
<tr>
<td>SWS</td>
<td>16.025 ± 5.56</td>
<td>15.431 ± 5.55</td>
<td>2.376 ± 1.77</td>
<td>4.033 ± 1.68</td>
<td>5.761 ± 2.87</td>
<td>17.437 ± 4.14</td>
</tr>
<tr>
<td>MWS</td>
<td>19.396 ± 9.75</td>
<td>25.909 ± 1.89</td>
<td>21.641 ± 4.05</td>
<td>8.528 ± 3.97</td>
<td>6.903 ± 1.87</td>
<td>10.801 ± 1.95</td>
</tr>
<tr>
<td>SUM</td>
<td>63.162</td>
<td>54.027</td>
<td>41.234</td>
<td>19.688</td>
<td>29.181</td>
<td>50.254</td>
</tr>
</tbody>
</table>

Table 2.1b Proportions of differences in photon catch (as percent reflectance [%]) between natural robin and the model eggs in each photoreceptor region across all model egg types and all photoreceptors. \( \Sigma^{2/\text{cone}} \) indicates the variance per cone across all model eggs. Proportion/cone indicates the proportion of total color variance that is available to each cone, across all model eggs.

<table>
<thead>
<tr>
<th></th>
<th>Yellow</th>
<th>Red</th>
<th>Blue</th>
<th>AMRO ground</th>
<th>UV-blocked</th>
<th>BHCO ground</th>
<th>( \Sigma^{2/\text{cone}} )</th>
<th>Proportion/cone</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVS</td>
<td>0.116</td>
<td>0.128</td>
<td>0.091</td>
<td>0.022</td>
<td>0.196</td>
<td>0.015</td>
<td>0.002</td>
<td>0.009</td>
</tr>
<tr>
<td>SWS</td>
<td>0.254</td>
<td>0.286</td>
<td>0.058</td>
<td>0.205</td>
<td>0.197</td>
<td>0.347</td>
<td>0.042</td>
<td>0.224</td>
</tr>
<tr>
<td>MWS</td>
<td>0.307</td>
<td>0.480</td>
<td>0.525</td>
<td>0.433</td>
<td>0.237</td>
<td>0.215</td>
<td>0.032</td>
<td>0.171</td>
</tr>
<tr>
<td>LWS</td>
<td>0.324</td>
<td>0.107</td>
<td>0.327</td>
<td>0.340</td>
<td>0.370</td>
<td>0.424</td>
<td>0.112</td>
<td>0.595</td>
</tr>
</tbody>
</table>
assess the effect of presentation order as a potential confound of egg rejection rates in response to sequential parasitism (e.g. Hauber et al. 2006; Samaš et al. 2011). The timing of experimental parasitism (day in the laying cycle that parasitism took place; day 1 = day of 1st egg laid) was also included as a possible predictor, as this is known to effect rejection rate (Welbergen et al. 2001; Moskat and Hauber 2007). Again, $\chi^2$ tests were used to examine effects of study year on acceptance/rejection, and Kruskal-Wallis tests were used to examine potential effects of egg color on the latency to rejection, defined as the number of days lapsing between experimental parasitism and egg rejection.

**Avian visual modeling effects of egg color on rejection**

To estimate differences between colors with respect to the spectral sensitivities of avian photoreceptors (Bennet and Thery 2007), we used the Vorobyev and Osorio (1998) model for tetrachromatic vision in AVICOL v5 software (Gomez 2010). American robins are known to be UVS (Chen et al. 1984; Chen and Goldsmith 1986; Aidala et al. 2012a), but physiological data for detailed spectral sensitivity of each photoreceptor were not available for our focal species. Therefore, we extracted spectral sensitivity data for the congeneric European blackbird, *T. merula,* from data published in Hart et al. 2000 using Vistametrix software (Vista Metrix 1.3, SkillCrest LLC, www.skillcrest.com) and ranging from 330 – 700nm. Because AVICOL requires sensitivity data ranging from 300 – 700 nm, we set photoreceptor absorbance for 300 – 330 nm to 0 (*sensu* Igic et al. 2010, 2012). Relative cone densities were set to UVS: 1, SWS: 2, MWS: 2, LWS: 2 (as listed for *T. merula;* Hart et al. 2000), and Weber fraction was set to 0.1 (Vorobyev et al. 1998). As the ability to discriminate different colors is influenced by environmental light
(Langmore et al. 2005; Munoz et al. 2007; Aviles 2008; Honza et al. 2011), we used published ambient light irradiance data for broken canopy forest (Vorobyev and Osorio 1998), which may most closely simulate the variable forest-edge light environments in which many American robins nest, even when breeding in sub/urban sites (Sallabanks and James 1999; RC pers. observ.).

AVICOL extracts quantum receptor catches for each single-cone receptor type, and combines these with the birds’ spectral sensitivities to quantify the birds’ ability to distinguish between any two colors as the perceptual distance between spectra ($\Delta S$) or as JNDs (“just noticeable differences”); JNDs exceeding 1.0 indicate a chromatic difference that is discriminable based on our estimates of avian spectral sensitivities (Osorio and Vorobyev 1996). AVICOL also extracts discriminability based on achromatic contrasts using the sum of the sensitivities of MWS and LWS cones, as these are similar to the sensitivities of rods and principal double-cone cells in the avian retina (Hart et al. 2000).

For the subsequent analyses, reflectance data for each natural robin egg was paired randomly with another (either wild or model) egg. We first evaluated the quality of our ‘mimetic’ model egg colors (i.e. AMRO ground, BHCO ground), designed to resemble natural eggs by comparing these to the natural eggs (cowbird or robin). We calculated mean JNDs distinguishing each natural egg type from its respective model, and compared these between groups using Welch’s two-sample t-tests, to evaluate whether one model egg better mimicked its natural counterpart. We also compared our model mimetic robin eggs with our UV-blocked model eggs in order to ensure that these
were perceptually discriminable based on avian visual sensitivities and therefore suitable for use in our analyses. We calculated mean JNDs differentiating these model egg types, and tested whether this differed statistically from 1 (as 1 JND signifies discriminable difference) using a one-sample t-test. As our UV-blocked and robin mimetic eggs differ only in the UV part of the spectrum and are discriminable based on avian vision ($\mu = 3.83$, $t = 3.17$, $df = 6$, $P = 0.019$; Figure 2.1), we examined the UV-blocked egg treatment as a test of differences specifically in the ultraviolet part of the avian visual spectrum.

We then calculated the difference between quantum receptor catches for real and model eggs across each of the four single-cone receptors. We summed these values within each pair, and then calculated the proportion of the total difference between eggs in each pair attributable to differences in each photoreceptor sensitivity region (normalizing photoreceptor catches across all four photoreceptors to equal 1 for each egg pair). Using these data, we tested the effect of proportionate differences in receptor catches on rejection rate across wild and model egg colors. To determine which avian photoreceptors contribute predictably to the rejection of foreign eggs, we used the Akaike Information Criterion (AIC) model selection approach (Burnham and Anderson 2002) to choose the best fit from among candidate photoreceptor models. Candidate models were derived by stepwise removal from an initial global logistic regression including proportionate differences in photoreceptor catches across each egg pair (as described above) and all possible interactions, with percent rejection (of eggs of a given model egg color) as dependent variable. The model with the lowest value of AIC provides the best balance between loss of precision due to overfitting and bias due to underfitting, and is
therefore the best fit model. Due to relatively small values for N and large values for K, we here report AICc values, AIC values corrected for finite sample sizes (N = 4 – 5 eggs’ reflectance spectra measured per color type in this analysis). The Akaike weights give the relative support for a given model compared with the other models in the set (Burnham and Anderson 2002). Analyses were conducted in R version 2.12.1.

Finally, for each model egg, we extracted the JND value differentiating model egg color spectra from natural robin egg spectra, as well as the pairwise achromatic contrast values. To test for effects of JNDs and achromatic contrasts on rate of rejection of foreign eggs, we fitted separate logistic regressions describing percent of model eggs rejected as a function of JNDs or achromatic contrast difference from natural robin eggs, across all model egg colors. When this regression analysis was significant (i.e. for JNDs but not for achromatic contrasts; see Results), we then calculated 95% confidence intervals (Mermoz and Ornelas 2004) around the rejection rates for each model egg type as predicted by JNDs differentiating that model egg color from the natural robin eggs. We plotted the positions (JND X Rejection %) of our artificial eggs, as well as the positions of natural (robin and cowbird) eggs (with experimental rejection data taken from Briskie et al. 1992), and examined whether these positions fell within the predicted 95% confidence range.
Results

**Egg rejection rates among model eggs**

Model egg color significantly predicted rejection rate (Kruskal-Wallis $\chi^2_6 = 25, P < 0.001$). Eggs dyed to resemble the ground color of cowbird eggs (“BHCO ground”, N = 10) were rejected in 100% of trials. Yellow eggs (N = 13) were rejected in 70% of trials. Red eggs (N = 14) were rejected in 64% of trials. Blue eggs (N = 15) were rejected in 58% of trials. UV-blocked eggs (N = 5) were rejected in 20% of trials. Eggs dyed to resemble the background color of robin eggs (“AMRO ground”, N = 7) were never rejected. Of the six model egg types presented, all were rejected at statistically similar rates except “AMRO ground” and “UV-blocked” eggs, which were rejected at significantly lower rates than all other model egg types (see Table 2.2 for pairwise comparisons and Fig. 2.2 showing 95% Wilson binomial confidence intervals for rejection rates).

Egg color did not significantly predict latency to rejection (Kruskal-Wallis $\chi^2_3 = 2.98, P = 0.394$; Fig. 2.3a). Presentation order and study year also did not statistically covary with rejection rates (presentation order, Kruskal-Wallis $\chi^2_4 = 4, P = 0.406$; year, $\chi^2_1 = 0.32, P = 0.569$; Fig. 2.3b,c). Likewise, the timing of parasitism within the incubation cycle was not significantly related to rejection rates (Kruskal-Wallis $\chi^2_8 = 8, P = 0.434$; Fig. 2.3d).
Table 2.2

Summary of the test statistics from paired color comparisons ($\chi^2$, from randomized outcome data). Asterisks denote significance among paired colors ($\alpha = 0.05$)

<table>
<thead>
<tr>
<th></th>
<th>BHCO ground</th>
<th>AMRO ground</th>
<th>Yellow</th>
<th>Red</th>
<th>Blue</th>
<th>UV-blocked</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHCO ground</td>
<td>$p = 0.0012^*$</td>
<td>$p = 0.5461$</td>
<td>$p = 0.3843$</td>
<td>$p = 0.2796$</td>
<td>$p = 0.0039^*$</td>
<td>$X^2 = 10.48$</td>
</tr>
<tr>
<td></td>
<td>$X^2 = 10.48$</td>
<td>$X^2 = 0.36$</td>
<td>$X^2 = 0.76$</td>
<td>$X^2 = 1.17$</td>
<td>$X^2 = 10.48$</td>
<td></td>
</tr>
<tr>
<td>AMRO ground</td>
<td></td>
<td>$p = 0.0003^*$</td>
<td>$p = 0.0041^*$</td>
<td>$p = 0.0057^*$</td>
<td>$p = 0.6152$</td>
<td>$X^2 = 0.25$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$X^2 = 12.86$</td>
<td>$X^2 = 7.64$</td>
<td>$X^2 = 8.00$</td>
<td>$X^2 = 0.25$</td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
<td>$p = 0.5468$</td>
<td>$p = 0.3129$</td>
<td>$p = 0.0027^*$</td>
<td>$X^2 = 9.03$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 0.36$</td>
<td>$X^2 = 1.02$</td>
<td>$X^2 = 9.03$</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.7400$</td>
<td>$p = 0.0074^*$</td>
<td>$X^2 = 7.17$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 0.11$</td>
<td>$X^2 = 7.17$</td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.0299^*$</td>
<td>$X^2 = 4.72$</td>
</tr>
<tr>
<td>UV-blocked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2.3 Non-significant relationships among a) model egg color and latency to rejection (days), b) presentation order and rejection (%) across all model egg types, 3) study year and percent rejection (%) across all model egg types, and 4) timing of parasitism (day in the incubation cycle, day 1 = 1st egg laid) and rejection (%) across all model egg types.
Avian visual modeling

Individual natural American robin eggs were, on average, discriminable from all model egg colors (for JNDs across all model egg colors, $P < 0.05$ relative to 1.0; one sample t-tests; Fig. 2.4). In particular, model eggs mimicking the ground color of natural robin eggs differed from natural robin eggs by a mean of 4.6 JNDs. Surprisingly, however, both model cowbird and, especially, natural cowbird eggs showed relatively low JND values against natural robin eggs (mean JNDs difference for model cowbird eggs = 5.51 JNDs; mean JNDs difference for natural cowbird eggs = 13.25), implying more avian-perceivable similarity between host and parasite eggs than previously appreciated (Friedmann 1929; Rothstein 1982).

**Fig. 2.4** Overall avian-perceivable chromatic (JNDs) and achromatic contrasts between natural robin and experimental model eggs. Colors listed beneath columns denote model egg colors as compared to natural robin eggs. Means ± standard errors are shown.

In contrast, our model eggs mimicking the background color of natural cowbird eggs differed from natural cowbird eggs by 26.7 JNDs. This was likely because natural cowbird eggs reflect strongly in the UV part of the spectrum (Fig. 2.1) yet
methodological constraints prevented us from mimicking reflectance in UV. When we also calculated JNDs between natural and model cowbird eggs after setting reflectance values between 300-400 nm to 0, JND values differentiating these eggs were reduced dramatically, to 5.6 JNDs. JNDs differentiating natural robin eggs from their model counterparts were significantly higher than JNDs differentiating natural and model cowbird eggs (Welch’s two-sample t test; $t_{52} = -3.53, P < 0.001$). This indicates that across the SWS, MWS, and LWS parts of the avian visual spectrum, our model cowbird eggs were closer in appearance to natural cowbird egg ground color than our model robin eggs were to natural model robin egg color. These calculations illustrate that using human-based (400-700 nm) wavelength sensitivity to design or assess color-similarity between host and parasite (including experimental) eggs is likely to result in misleading levels of avian-perceivable similarity.

The model best predicting rejection rate for model eggs supports the consistent role of differential photoreceptor catch across the entire avian spectral sensitivity range in eliciting egg rejection. This model included terms for UVS, SWS, MWS, and LWS photoreceptor catches, and their interaction terms (Table 2.3).
Table 2.3

Summary of the differences among the candidate models with AICc weights summing to 1, chosen by Akaike Information Criteria with stepwise removal. The best fit model has the lowest value of AICc and highest AICc weight (Wt). UVS, SWS, MWS, and LWS refer to single-cone photoreceptor types ultraviolet-sensitive, short-wavelength sensitive, medium-wavelength sensitive, and long-wavelength sensitive, respectively.

<table>
<thead>
<tr>
<th>Model</th>
<th>Res. df</th>
<th>K</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>AICc Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVS + SWS + MWS + LWS + UVS<em>SWS + UVS</em>MWS + SWS<em>MWS + UVS</em>LWS + SWS<em>LWS + UVS</em>SWS*LWS</td>
<td>18</td>
<td>12</td>
<td>285.18</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td>UVS + SWS + MWS + LWS + UVS<em>SWS + UVS</em>MWS + SWS<em>MWS + UVS</em>LWS + SWS<em>LWS + MWS</em>LWS + UVS<em>SWS</em>LWS</td>
<td>19</td>
<td>13</td>
<td>291.22</td>
<td>6.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Finally, avian visual modeling also revealed that JND values significantly predicted rejection rates across model egg colors (logistic regression, $t_{28} = 2.12$, $P = 0.044$; Fig. 2.5). In contrast, achromatic contrast values did not significantly predict rejection rates (logistic regression, $t_{28} = 1.09$, $P = 0.287$; Fig. 2.4, bivariate plot not shown). The 95% confidence interval surrounding rejection rates, as predicted by JNDs includes rejection rates for yellow, red, and blue model egg colors. In contrast, rejection rates for robin ground color model eggs and UV-blocked eggs fall below the 95% confidence interval threshold, as these eggs are rarely rejected (robin ground = 0% rejection, UV-blocked = 20% rejection), despite their appreciable avian-perceivable discriminability from natural robin eggs (Fig. 2.4). Natural robin eggs fall well below the 95% confidence interval, and these are never rejected (Briskie et al. 1992). Model eggs mimicking the background color of cowbird eggs fall well above the 95% confidence interval, as they are rejected in 100% of experimental trials (Fig. 2.5). Likewise, natural
cowbird eggs fall well above the 95% confidence interval and are always rejected (Briskie et al. 1992).

**Fig. 2.5** Bivariate scatterplot of mean JNDs difference between natural robin eggs and each egg type plotted against the rejection (%) for that respective egg type. Shaded area represents 95% confidence interval based on the best fit logistic regression (fit line) for model eggs used in this experiment. Egg rejection rates of experimentally introduced, natural robin or cowbird, eggs were taken from Briskie et al. (1992)
Discussion

In American robins, the likelihood of rejection of model eggs dyed with various artificial colors, spanning the full range of avian-visible light, is best predicted by a model containing quantum photoreceptor catches of all four avian photoreceptors. Likewise, overall avian-perceivable chromatic difference between natural and model eggs (JNDs) significantly predicts rates of rejection. In support of hypothesis (i), these results imply that model egg colors perceived as more different from the robins’ own eggs will be rejected at higher rates.

More critically, visual modeling revealed that our mimetic experimental robin eggs were predicted to be perceptually discriminable from natural robin eggs (JNDs > 1.0 threshold for our mimetic model eggs) yet these were never rejected (Figs 2.4, 2.5). This may reflect a caveat in our perceptual modeling methodology, as we modeled avian vision using the photoreceptor sensitivities of congeneric European blackbirds, rather than of American robins per se, which are not yet available. In turn, cowbird eggs, whether model or natural (Rothstein 1982; Briskie et al. 1992; this study) are typically always rejected, despite the relatively low overall avian-perceived discriminability from natural robin eggs (Figs 2.4, 2.5). The latter pattern of unpredictably high rejection rates of cowbird eggs is in support of our hypothesis (ii). Coevolution with cuckoos and cowbirds, then, may have shaped the robins’ visual system in ways which cannot be predicted from our visual modeling approach and/or the use of known visual physiology of T. merula. Statistical techniques now exist to detect sensory coevolution between single pairs of host-parasites within a set of multispecies comparative analyses (Anderson
et al. 2009), but these would require detailed new anatomical and physiological studies of the robin’s actual visual system, perhaps at the level of individual variation (Fernández-Juricic et al. 2013). Further research should also address potential differences in photoreceptor evolution between hosts of mimetic versus nonmimetic brood parasites by modeling spectral sensitivities of American robins, and comparing these to those of species within the species rich lineage of the closely related European *Turdus* thrush clade (Voelker et al. 2007).

That mimetic model robin eggs were discriminable from natural eggs and yet were never rejected may demonstrate that robins tolerate some degree of color difference within their clutch. Behaviors otherwise indicating the detection of a parasitic egg, which are not then followed by egg rejection, have been documented in song thrush (Honza et al. 2007), yellow warblers (*Setophaga petechia*; Guigueno and Sealy 2012), great reed warblers (*Acrocephalus arundinaceous*; Moskat and Hauber 2007), and eastern olivaceous warblers (*Hippolais pallida*; Antonov et al. 2009). This is consistent with the suggestion that there exists a plastic, perceptual and/or cognitive threshold for rejection that is separate from that of visual discrimination; alternatively, this may result from plasticity in host acceptance threshold in response to context of parasitism (Hauber et al. 2006; but see Vikan et al. 2009). Either case implies that egg rejection decisions are not wholly governed by limitations of the sensory and perceptual systems (de la Colina et al. 2012). Future work should also reconcile the differences between the sensory models and the observed behavioral thresholds of egg discrimination vs. rejection, including the
possibility that robins perceivably discriminate between more eggs than they reject (see Moskat and Hauber 2007; Antonov et al. 2009).

Using a combination of artificial egg colors spanning the full range of the avian-visible spectrum, and the specific UV-blocking treatment in our experiments, these data also allow us to assess the role of UV-matching (Cherry and Bennett 2001) in eliciting egg rejection. In contrast to hypothesis (iii), we suggest that egg rejection in robins is not driven specifically by differences in the ultraviolet part of the avian visual spectrum, because UV-blocked model eggs were rejected at low rates, which were statistically similar to rejection rates for our model robin mimetic eggs and lower than all other colors of model eggs. Similarly, rejection rates for both fell below the 95% confidence interval for predicted rejection based on their respective JNDs (Table 2.2, Fig. 2.5). Cherry and Bennett (2001) posited that eggs appearing dissimilar to humans may actually appear similar in the ultraviolet part of the spectrum not visible to humans. For song thrush, congeneric with the robin, egg rejection is elicited by differences in photoreceptor catches for the UVS and SWS photoreceptors (Cassey et al. 2008). Because song thrush are parasitized, if rarely, by common cuckoos (Grim 2006), an egg-removing parasite and a member of a violet- (not UV) sensitive parasitic lineage (Mullen and Pohland 2008; Aidala et al. 2012b), differences in UV reflectance may benefit hosts by allowing for discrimination between their own and parasitic eggs without conferring that same advantage on the parasite (but see Aviles et al. 2005). As both robins and cowbirds are predicted to be UV-sensitive (Parrish et al. 1984; Aidala et al. 2012a), this selective advantage would not exist in the latter host-parasite system. UV reflectance varies widely
among cowbird host species’ eggs (Underwood and Sealy 2008), yet there is very little variation in host responses to cowbird parasitism, in that most hosts are either strong rejecters or acceptors of natural or artificial cowbird eggs (Takasu 1998). Thus, UV chroma is unlikely to act as a particular cue for egg rejection by cowbird hosts, including robins (Underwood and Sealy 2008); this suggestion is here supported not only by our rejection data from the experiments with robin ground and UV-blocked eggs, but also by the detailed visual analyses which did not highlight a disproportionate role for UVS and SWS receptors in predicting egg rejection rates across different model egg colors (Table 2.3).

The results of this study indicate that egg rejection in robins occurs in response to overall differences in color across the entire avian visual spectrum, including quantum receptor catches from all four avian single-cone photoreceptors, and not limited to input from UV-sensitive photoreceptors. However, all cowbird eggs are rejected by this host, despite relatively high overall avian-perceived similarity to the robins’ own egg color. This suggests that robins respond specifically to parasitism by cowbirds, despite an apparent lack of sensory tuning toward detection of cowbird eggs. Further study should investigate both the nature and extent of selective pressures on the sensory, cognitive, and behavioral mechanisms of egg-rejection by American robins in response to parasitism by brown-headed cowbirds.
Ethical Standards

This study was conducted on private land with the express permission of landowners and following the protocols and permissions of institutional and governmental agencies. The protocol was approved by the Institutional Animal Care and Use Committee of Hunter College (# MH 2/13-T3).

Acknowledgements

For financial support we thank the CUNY Graduate Center, American Ornithologists’ Union, Animal Behavior Society, the PSC-CUNY grant scheme, and the Human Frontier Science Program. For discussions and assistance we thank Zachary Aidala, Jennifer Basil, Phill Cassey, Tomas Grim, Brani Igic, David Lahti, Lisa Manne, Csaba Moskat, Lainga Tong, Michael Webster, and Sarah Woolley.
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CHAPTER 3

A recoverable cost of brood parasitism during the nestling stage of the American robin (*Turdus migratorius*): implications for the evolution of egg rejection behaviors in a host of the brown-headed cowbird (*Molothrus ater*)

Rebecca Croston1*, Mark E. Hauber1,2

1 Ecology, Evolutionary Biology, and Behavior Subprogram in Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10016, USA

2 Department of Psychology, Hunter College and The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10065, USA

* Corresponding author: RCroston@gc.cuny.edu, Hauber Lab, Dept. Psychology, Hunter College, 695 Park Avenue, New York, NY 10065, USA, +1 (212)-396-6445.

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ABSTRACT

Hosts of brood parasitic birds face reduced reproductive success as a direct consequence of rearing parasitic young. The most commonly evolved host behavior to combat costly parasitism is the rejection of foreign eggs. Despite consistent patterns of reduced nesting success in broods parasitized by brown-headed cowbirds (*Molothrus ater*), most of its host species do not reject foreign eggs. Paradoxically, where it is present, egg rejection is more common among cowbird hosts with larger body sizes, yet cowbird chicks are less likely to outcompete larger host nest mates. This raises a fundamental question whether egg rejection, even in the minority of cowbird hosts, has specifically evolved in response to costly brood parasitism. Here we tested predictions associated with this hypothesis in an egg-rejecter host, the American robin (*Turdus migratorius*), by assessing whether experimental cowbird parasitism causes reduced nesting success. We cross-fostered cowbird and control, host chicks into robin nests, testing for experimental effects of chick species, brood size, and hatching asynchrony; unmanipulated nests served as additional controls. Rearing a foreign chick in the brood reduced the reproductive output of host robins, however, we detected no effect of parasitizing host nests with a cowbird versus robin chick. Cowbird chicks were significantly less likely to fledge than cross-fostered robin chick controls. These experiments reveal that parasitism itself can exert a cost on robin hosts during the nestling stage, representing a recoverable cost of cowbird parasitism that can be avoided by the host through rejecting parasite eggs prior to hatching. These results support the assumption that foreign egg rejection is an evolved host response to brood parasitism in this system, despite the poor survival rate of cowbird chicks in robin broods.
KEY WORDS: American robin, brood parasitism, brown-headed cowbird, cross-fostering,

*Molothrus ater*, survivorship, *Turdus migratorius*
INTRODUCTION

Hosts of brood parasitic birds face a reduction in fitness, typically experienced as reduced nesting success when rearing genetically unrelated young (Payne 1977, 2005; Rothstein 1990; Slagsvold 1998; Davies 2000; Hauber 2003a; Kilner 2003). The costs of brood parasitism range from reduced hatching of host eggs (Rothstein 1975; Hauber 2003b) to lower fledging success due to competition with or killing by parasitic offspring (Friedmann 1929; Lorenzana & Sealy 1999; Rutila et al. 2002; Grim et al. 2009; Spottiswoode & Koorevaar 2012). For example, brown-headed cowbirds (*Molothrus ater*) do not directly destroy or displace host nestlings or eggs (Dearborn 1998), and instead hosts typically experience reduced fledging success due to rearing a more competitive and earlier hatching parasite alongside host chicks (Rothstein 1975; Sedgwick & Iko 1999; Hauber 2003a). In response to costly obligate brood parasitism, hosts have evolved diverse defense portfolios (Davies 2011; Kilner & Langmore 2011), including egg rejection (Friedmann 1929; Davies & Brooke 2009) and/or the desertion of parasitized clutches or broods (Hosoi & Rothstein 2000; Moskat et al. 2011), as well as defending the nest against egg-laying by the adult female parasite (Sealy et al. 1998; Feeney et al. 2012).

Despite decreased nesting success associated with brown-headed cowbird (hereafter: cowbird) parasitism, most hosts accept foreign eggs and rear cowbird chicks alongside their own (Lorenzana & Sealy 1999; Hauber 2003a; Kilner 2003). Typical cowbird hosts either abandon parasitized broods (Hosoi & Rothstein 2000) or accept parasitism and raise mixed broods (Payne & Payne 1998). In contrast, American robins...
(Turdus migratorius; hereafter, robin) are able to recognize and reject cowbird eggs in response to natural or experimental parasitism (Rothstein 1982; Briskie et al. 1992).

For egg rejection to have evolved specifically as an adaptive response to cowbird parasitism, tolerating cowbird eggs in a clutch and/or rearing cowbird hatchlings must impose recoverable costs on robins as hosts. Recoverable costs of parasitism are those that are not associated with parasitic egg laying itself, that is, those that are reduced or eliminated when a cowbird egg is removed from the nest (Røskaft et al. 1990; Payne & Payne 1998; Lorenzana & Sealy 2001; Anderson et al. 2009). Cowbird nestlings hatch earlier, and are typically larger and more competitive than those of their hosts (Friedmann 1929; Hauber 2003c). Cowbird nestlings thus reduce the fledging success of most hosts by outcompeting host chicks and monopolizing parental feedings (Dearborn 1998; Hauber 2003b; Kilner et al. 2004), resulting in reduced host chick growth and survival, especially for later hatching and smaller cowbird hosts (Payne & Payne 1998; Kilner 2003; Hauber 2003a).

Paradoxically, egg ejector status of cowbird hosts is positively correlated with body size (Rothstein 1975; Rohwer & Spaw 1988; Røskaft et al. 1993; Peer & Sealy 2004). However, large cowbird hosts generally sustain little to no cost associated with rearing a cowbird chick alongside their own young (Lorenzana & Sealy 1999; Hauber 2003a). This raises the fundamental question whether egg rejection in cowbird hosts has specifically evolved in response to interspecific brood parasitism. Here we test predictions stemming from the hypothesis that egg rejection in robins evolved in defense of cowbird parasitism, by assessing whether rearing a parasitic chick decreases host hatching and fledging success.
While no previous studies have addressed the cost to relatively large and rapidly developing *Turdus* hosts parasitized by *M. ater*, the presence of a smaller parasitic shiny cowbird (*M. bonariensis*) chick had no significant detrimental effect on survival or growth rate in two larger hosts’ nestlings: the creamy-bellied thrush (*T. amaurochalinus*; Astie and Reboreda 2006) and rufous-bellied thrush (*T. rufiventris*; Sackmann & Reboreda 2003). American robins hatch from a larger egg at a larger size, and within just few days of brown-headed cowbirds eggs and hatchlings (Lorenzana & Sealy 1999; Hauber 2003a), and develop more rapidly relative to other cowbird hosts (Lowther 1993). Because of this, cowbird chicks may fare poorly due to intensive competition with host robin chicks. Additionally, robin parents may forego direct costs of feeding foreign chicks by providing more frequent or higher quality food to their own chicks (as seen in other *Turdus* thrushes: Lichtenstein 2001; Soler 2008; Grim et al. 2011), resulting in variation among individual nestlings in growth rate and condition (Hauber & Kilner 2007). Conversely, though, cowbird chicks have adaptations to be more responsive to parental feeding cues than host chicks (Hauber 2003c), and thus may yet outcompete robins, especially if the earlier presence of the cowbird chick in the nest interferes with ongoing incubation or hatching of the host clutch (Hauber 2003b). Alternatively, the earlier presence of the cowbird chick may cause the robin parents to switch feeding from regurgitated to solid food (Tyler 1949, as cited in Sallabanks & James 1999) prematurely for the later hatching robin chicks, causing poor nutrition and/or starvation of their own chicks. As these are empirical questions, and because robins naturally reject nearly all cowbird eggs from their nests, making natural co-rearing of robins and cowbirds rare, we conducted an experiment to assess quantitatively the extent of cowbird and host hatching.
and fledging success. We evaluate the potential cost of cowbird parasitism by testing whether rearing a parasitic nestling alongside hosts’ own young reduces hatching and fledging success in robin nests.

METHODS

Study site and manipulation

Data collection took place in and around Ithaca, NY, USA, throughout the breeding seasons of 2010-2012. As robins are highly commensal and often nest in and around human structures (Sallabanks & James 1999), nests were located by searching in and around residential areas, as well as in wooded areas surrounding human habitation. Upon finding a robin nest containing at least one egg, we monitored nesting activity in order to assess the time of hatching. We also monitored nests of egg-accepting cowbird hosts as available at our study site, including nests of the eastern phoebe (Sayornis phoebe), veery (Catharus fuscuscens), and wood thrush (Hylocichla mustelina). These nests were used as sources of known-age cowbird chicks, which were removed after hatching and placed in robin nests (see below). Where suitable robin nests were available, 1 host chick was removed from nests where 4 robin eggs/chicks were present (N = 6 replacements). This was done in order to avoid experimental brood sizes > 4, the maximum at unmanipulated robin nests in our study population (Croston and Hauber in press). For nests with no alternate available to place removed chicks, parasitic chicks were added without removal of a host chick (N = 19). These nests were considered a ‘parasitic’ chick addition treatment rather than a replacement treatment. Treatment
groups were assigned solely based on availability as determined by the timing of hatching, therefore we did not have a priori expectations of a confound between genetic and environmental factors.

We moved cowbird chicks into robin nests following one of three treatments, with parallel control manipulations with cross-fostered conspecific chicks: 1) host/parasite same-age (N = 10), 2) parasite 3 days older than host, post-hatch of host clutch (N = 5), 3) parasite 3 days older than host, pre-hatch of host clutch (N = 10). A 4th, control group of robin nests remained unmanipulated regarding brood composition, serving as biological and methodological controls (N = 25). By including both same-age and older cross-fostered eggs or chicks, we aimed to disentangle the effects of species identity from those of hatching interference or asynchrony (Hauber 2003b). Cowbird eggs hatch after approximately 10 days of incubation, and robins after 12-14 days (Lowther 1993; Sallabanks & James 1999; R. Croston pers. obs.), and our treatment mimicked the natural 3-day hatching asynchrony that would result from parasitism were a cowbird egg to remain in a robin nest until hatching, given a mean 13-day incubation period for robins (Sallabanks & James 1999, R. Croston pers. obs.).

We monitored manipulated nests every second day until fledging (robins: 13 days after hatching, Sallabanks & James 1999; cowbirds reared by robins: 10 days after hatching, Hauber 2003a; R. Croston pers. obs.). We tracked the number of eggs hatching and chicks fledging using hand-held mirrors and/or binoculars as necessary to visually confirm the presence and number of eggs and chicks. To avoid force-fledging, we did not approach nests after day 11 post-hatch. Robin chicks present in the nest at day 11 post-hatch were assumed to have fledged successfully from nests found to be empty upon next
visit. We inspected and collected the same clutch and brood size metrics from our control nests: non-parasitized robin nests monitored at our study site (N = 25) during the same breeding seasons as our experimental manipulations.

Statistical analyses

Because one of the predicted outcomes of our treatments was reduced survival of host chicks, we limited our sample size (totaling N = 50 nests) in order to avoid imposing unnecessary negative impact on our subject population, without limiting our ability to answer our critical questions. We first calculated proportions of host eggs hatched from eggs laid, and host chicks fledged from both eggs hatched and eggs laid in each experimental nest by dividing the number of eggs hatching into the number of eggs laid (“egg survivorship”), the number of chicks fledging into the number eggs hatching (“chicks fledged from eggs hatched”), and the number of chicks fledging into the number of eggs present in the nest prior to hatching (“chicks fledged from eggs laid”). Egg survivorship is a mechanistic reflection of differential survivorship specifically at the egg and hatching stage, and the proportion of chicks fledged from eggs hatched reflects differential survivorship as a result of interference specifically at the chick stage (Hauber 2000). The proportion of chicks fledged from eggs laid is a proxy for overall fitness, as it reflects reproductive return on initial reproductive investment.

To examine the effects of hatching asynchrony, nest stage at introduction (egg vs. chick), brood size increase, and ‘parasitic’ species identity (cowbird/robin) of the host chicks, we constructed separate generalized linear mixed models (GLMMs) with each of the three host survivorship metrics as response variables, and chose among successive
candidate models using AIC model selection (Burnham & Anderson 2002) with backward stepwise removal. The global model predicting egg survivorship contained only year as a random effect and Julian date, its quadratic term to allow for non-linear seasonal peak effects (sensu Samaš et al. 2013), and cross-fostered species as fixed effects. This is because egg survivorship treatment was necessarily the addition of a 3 days older chick at the egg stage, therefore there could be no meaningful variation among these predictors. The global models predicting the proportion of chicks fledged from eggs hatched, and proportion of chicks fledged from eggs laid contained year as a random variable and Julian date, its quadratic term, cross-fostered species, age difference, nest stage, and brood size increase as predictors. Similarly, we constructed a full model for parasitic chick survival to fledging as a binomial generalized linear mixed model (GLMM) and chose parameters from the global model including Julian date, its quadratic term, hatching asynchrony, nest stage, brood size increase, and cross-fostered species identity (cowbird/robin) using backward stepwise removal.

We fit additional global models predicting effects of manipulation across our 3 host survivorship metrics between the sum set of all manipulated nests and the set of unmanipulated nests. Here, year was included as a random effect, and treatment group (manipulated/unmanipulated) was included as a fixed effect.

We conducted post-hoc tests investigating specific effects of each experimentally manipulated fixed effect remaining in our best fit models, as chosen based on AICc values (see Table 3.1). We examined the specific effects of cross-fostered chick species on egg survivorship, using a Kruskal-Wallis test. Next, we tested effects of cross-fostered species, age difference, nest stage at parasitism, and addition versus replacement
of the ‘parasitic’ chick on the proportion of chicks fledged from eggs hatched, and proportion of chicks fledged from eggs laid with separate Kruskal-Wallis tests. We examined the effect of chick species on the survival of cross-fostered chicks themselves, using a \( \chi^2 \) test. Finally, we pooled data across all manipulated nests, and compared host survivorship metrics between manipulated and unmanipulated nests using Kruskal-Wallis tests.

All analyses were conducted in R version 2.15.2, using additional packages lme4 and MuMIn.

**RESULTS**

For egg survivorship, GLMM with stepwise removal yielded a best fit model containing terms for Julian date and parasitic chick species. Similarly, the best fit models predicting both the proportion of chicks fledging from eggs hatched and the proportion of chicks fledging from egg laid contained terms for cross-fostered species, age difference, nest stage, and brood size increase. Our best fit model predicting survivorship of the cross-fostered chicks themselves contained cross-fostered species identity, Julian date, and its quadratic term. Models are summarized in Table 3.1.

Our tests for effects of manipulation on egg survivorship yielded best fit models for all survivorship metrics which contained the term for manipulated versus unmanipulated nests. These results are summarized in Table 3.2.
Table 3.1. Summary of model parameters for the best fit models describing each of the host survivorship metrics, and cross-fostered chick survivorship, and the second best fit models. Best fit models were selected based on AIC model selection with backward stepwise removal. Variables listed in the table were included as fixed effects. All models contain Year as a random effect. Global models also contained a quadratic Julian date term (“Julian date^2”), which accounted for any peak in fledging success in the middle of the breeding season. “Add/Rep” represents brood size increase (Addition/Replacement). For each variable, the estimate’s mean and (S.E.) as calculated from GLMM is listed. NA indicates that a term was not entered into the full model (see Methods).

<table>
<thead>
<tr>
<th>Response</th>
<th>Model parameters</th>
<th>Julian date</th>
<th>Julian date^2</th>
<th>Species</th>
<th>Age dif.</th>
<th>Stage</th>
<th>Add/Rep</th>
<th>df</th>
<th>AICc</th>
<th>Δ</th>
<th>w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg survivorship</td>
<td>Best fit</td>
<td>-0.16 (0.30)</td>
<td>-13.26 (14.73)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>235.2</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>- 1</td>
<td>-0.01 (0.25)</td>
<td>-13.26 (14.73)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4</td>
<td>240.1</td>
<td>4.87</td>
<td>0.08</td>
</tr>
<tr>
<td>Chicks fledged from eggs hatched</td>
<td>Best fit</td>
<td>8.33 (18.82)</td>
<td>13.83 (25.09)</td>
<td>-27.64 (25.92)</td>
<td>22.22 (19.16)</td>
<td>7</td>
<td>170.6</td>
<td>0</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 1</td>
<td>11.06 (23.63)</td>
<td>-23.01 (23.08)</td>
<td>20.37 (18.19)</td>
<td>6</td>
<td>173.3</td>
<td>2.70</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicks fledged from eggs laid</td>
<td>Best fit</td>
<td>13.57 (19.66)</td>
<td>-5.20 (26.21)</td>
<td>-13.19 (27.08)</td>
<td>28.98 (20.02)</td>
<td>7</td>
<td>171.8</td>
<td>0</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 1</td>
<td>-9.72 (24.93)</td>
<td>-5.66 (24.34)</td>
<td>25.96 (19.19)</td>
<td>6</td>
<td>174.9</td>
<td>3.08</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-foster fledged (Y/N)</td>
<td>Best fit</td>
<td>3144 (3.72*10^5)</td>
<td>7.71 (908.90)</td>
<td>-2.77<em>10^4 (3.27</em>10^4)</td>
<td>5</td>
<td>17.2</td>
<td>0</td>
<td>0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 1</td>
<td>-0.36 (0.42)</td>
<td>-22.26 (25.54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Table 3.2. Summary of model parameters for the best fit models describing each of the host survivorship metrics in response to nest manipulation, as chosen based on AIC model selection with backward stepwise removal. All models contain Year as a random effect and manipulated/non-manipulated as a fixed effects. For each variable, the estimate’s mean and (S.E.) as calculated from GLMM is listed. Across all 3 survivorship metrics, whether or not a nest was experimentally manipulated remained in the best fit model. Survivorship was lower in manipulated nests across all 3 metrics.

<table>
<thead>
<tr>
<th>Response</th>
<th>Model Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manip/Non-manip</td>
</tr>
<tr>
<td>Egg survivorship</td>
<td>6.36 (6.13)</td>
</tr>
<tr>
<td>Chicks fledged from eggs hatched</td>
<td>19.31 (6.58)</td>
</tr>
<tr>
<td>Chicks fledged from eggs laid</td>
<td>18.07 (7.88)</td>
</tr>
</tbody>
</table>
Tests of host survivorship

Our post-hoc tests revealed that cross-fostered chick species (cowbird/robin) had no significant effect on host egg survivorship (Kruskal-Wallis $\chi^2 = 0.26$, df = 1, $P = 0.61$; Fig. 3.1a). Cross-fostered chick species had no significant effect on chicks fledged from eggs hatched (Kruskal-Wallis $\chi^2 = 0.18$, df = 1, $P = 0.67$), or chicks fledged from eggs laid (Kruskal-Wallis $\chi^2 = 0.010$, df = 1, $P = 0.92$, Fig. 3.1a). Experimental hatching asynchrony and nest stage at introduction (host pre-hatch vs. host post-hatch) likewise had no significant effects (Kruskal-Wallis tests: hatching asynchrony, chicks fledged from eggs hatched: $\chi^2 = 0.02$, df = 1, $P = 0.89$, chicks fledged from eggs laid: $\chi^2 = 1.06$, df = 1, $P = 0.30$, Fig. 3.2a; nest stage at introduction, chicks fledged from eggs hatched: $\chi^2 = 0.15$, df = 1, $P = 0.70$, chicks fledged from eggs laid: $\chi^2 = 0.34$, df = 1, $P = 0.55$).

We also found no significant effect of cross-fostered chick addition versus replacement on host nesting success (Kruskal-Wallis tests, chicks fledged from eggs hatched: $\chi^2 = 2.44$, df = 1, $P = 0.12$, chicks fledged from eggs laid: $\chi^2 = 1.05$, df = 1, $P = 0.31$, Fig. 3.2b).

Tests of parasitic chick survivorship

For the parasitic chicks themselves, we found significant differences in survivorship based on species, such that cross-fostered cowbird chicks are significantly less likely to survive to fledging than are cross-fostered ‘parasitic’ conspecifics (Kruskal-Wallis test, $\chi^2 = 4.82$, df = 1, $P < 0.05$; Fig. 3.1b).
Figure 3.1
(A) Variation in the proportions of egg survivorship, chicks fledged from eggs hatched, and chicks fledged from eggs laid per clutch and brood, between nests experimentally parasitized with robin versus cowbird chicks. Error bars denote standard error as calculated from GLMM. None of the comparisons showed a statistically significant difference. (B) Variation in the proportion of fostered parasitic chicks surviving to fledging. Asterisk denotes significant difference.

Comparison across manipulated versus unmanipulated robin nests yielded no significant differences in egg survivorship or proportion of chicks fledged from eggs laid (Kruskal-Wallis test, egg survivorship: $\chi^2 = 0.24, df = 1, P = 0.62$, proportion chicks fledged from eggs laid: $\chi^2 = 2.73, df = 1, P = 0.10$; Fig. 3.3). The proportion of chicks fledged from eggs hatched was, however, significantly lower in manipulated than unmanipulated nests (Kruskal-Wallis test, $\chi^2 = 7.91, df = 1, P < 0.01$; Fig. 3.3), indicating that experimental brood parasitism, irrespective of hatching asynchrony (3 d/none), stage (egg/nestling) and fostered species (cowbird/robin), imposed a cost on host nesting success, but only during the nestling stage.
Figure 3.2

(A) Variation in the proportions of chicks fledged from eggs hatched, and chicks fledged from eggs laid per clutch and brood between nests where hosts and parasite chicks were the same age/stage (“Synchronous”) versus nests where parasitic chicks were 3-days-older than host chicks (“Asynchronous”). (B) Variation in the proportions of chicks fledged from eggs hatched, and chicks fledged from eggs laid per clutch and brood between nests where ‘parasitic’ chicks were added ("addition") to existing nest contents versus exchanged ("replacement"). Error bars denote standard error as calculated from GLMM. None of the illustrated comparisons showed a statistically significant difference.
Variation across all measures of host nesting success per clutch and brood between manipulated and unmanipulated (control) robin nests. Error bars denote standard error as calculated from GLMM. Asterisk denotes significant difference.

DISCUSSION

Our experiments revealed no statistically significant reduction in the overall reproductive success of robins as a result of co-rearing cowbird young alongside their own progeny, and irrespective of the brood size, hatching success, or the stage of introduction (egg/nestling) of the foreign chick. In contrast, there was statistical support for the reduced survival of cross-fostered cowbird nestlings relative to cross-fostered robin chicks, confirming predictions that cowbirds, as small brood parasitic young, fare poorly in the brood of this large host species (Kilpatrick 2002; Kilner 2003). Critically, however, we also detected a cost of parasitism itself during the nestling stage, such that robins fledged fewer chicks from those which hatched successfully in any experimentally
parasitized nest, relative to unmanipulated broods. Given that this statistical pattern was not replicated in two other measures of nesting success, which encompassed a broader stage of the robin nesting cycle, we conclude that these specific results were not an experimental artifact and represent biological reality. This finding, therefore, suggests that despite their poor survival in this host’s broods, cowbirds may exert selective pressure on robins at the nestling stage, which can be avoided by rejecting the cowbird egg prior to hatching. The rejection of foreign eggs, therefore, is likely a specific adaptation of robins in response to brood parasitism by cowbirds.

We specifically assessed the extent of recoverable cost of parasitism (Hauber 2003a), and, together with the unrecoverable cost of parasitism linked with the parasite’s removal of host eggs (Hauber 2003d; Servedio & Hauber 2006), it is likely that the cost of cowbird parasitism is even greater in robins than that measured here. We also did not account for recognition and rejection costs associated with attempts to reject parasitic eggs in our study, although Rasmussen et al. (2009) found no such cost for robins in a different population. Sackmann and Reboreda (2003) examined costs associated with parasitism in two large hosts of shiny cowbirds *M. bonariensis*, the chalk-browed mockingbird *Mimus saturninus* and rufous-collared thrush (*T. rufitorques*), and found that the only cost of parasitism for these hosts is the cost of egg puncture by the cowbirds coincident with parasitism. Future work should involve video monitoring of robin nests in order to account for both egg removal and/or puncture by cowbirds during parasitism events, and rejection errors leading to loss of hosts eggs (*sensu* Lorenzana & Sealy 2001).

That cross-fostered cowbird chicks were less likely to survive to fledging in robin nests (50%) than were cross-fostered robin chicks (90%) may indicate that robins are
poorly suited hosts for brood parasitic cowbirds. Cowbird chicks may survive poorly in the nests of host robins due to unsuitable diet (Davies 2000; Yang et al. 2013); specifically, robin parents switch from feeding chicks with regurgitated to whole food around the 4th day (Tyler 1949, from Lowther 1993), and nestlings may be provisioned with up to 30% plant materials (Howell 1942, from Lowther 1993). Low cowbird survivorship in robin nests may be due in part to dietary differences (Grim et al. 2011; Yang et al. 2013). Additionally, dietary mismatch could arise from differences in manageable prey size between this parasite and host, where prey that is typically fed to host chicks is too large for the smaller cowbird chick to handle (Peer & Bollinger 1997).

Alternatively, the cowbirds’ lower success in robin nests may result not from unsuitable diet, but as a competitive byproduct of the dramatic difference in size between robin and cowbird nestlings. Cowbird chicks survive best in host nests with 1-2 nestmates co-habiting the brood (Kilner 2003). In robins, even if the cowbird female removes a host egg, the inability of the cowbird chick to consistently reduce the host’s own brood size means that 2-3 large robin chicks would typically be raised together with the smaller parasite; this is suboptimal for cowbird chicks and would explain the lower survival rate (also see Kilner et al. 2004).

Mechanistically, robin parents provide the most provisioning to chicks reaching up highest in the nest and bringing their bill closest to that of the parent (McRae et al. 1993). As parents consistently land on certain portions of the nest rim, chicks situated in certain sectors of the nest consistently receive more provisioning. This results in competition among robin chicks by ‘jockeying’ for position within the nest cup (McRae et al. 1993). Robins hatch at approximately 4 times the mass of cowbirds
(Lowther 1993; Sallabanks & James 1999; R. Croston, upubl. data), and at fledging, robin chicks have an average mass of ~55g, whereas cowbird chicks reared by robins average ~30g (R. Croston, upubl. data). We found no effect of hatching asynchrony on nestling survivorship, indicating that the cowbirds’ shorter incubation time does not provide cowbird chicks with a competitive advantage. Future research should assess the importance of the difference in nestling size in the cowbird-robin system, relative to competitive asynchrony, in jockeying for favorable positions within the nest to solicit parental provisioning (sensu Hauber 2003a), leading to the reduced growth and starvation of parasitic chicks.

Here, nearly 50% of cowbird chicks failed to fledge when reared by robins (Fig. 1). This suggests that robins may be unsuitable relative to other cowbird hosts, where cowbird chick mortality is generally low (typically less than 50%, Kilner 2003). Using the same metrics calculated by Hauber (2003), robins pay a lower cost of cowbird parasitism than other host species (Hauber 2003).

Brood parasite chicks are poor competitors with European Turdus thrush nestmates in cross-fostering experiments involving European species. When nest cup design prevented successful host egg ejection by the hatchling parasite, parasitic common cuckoo young were unable to compete with the large, rapidly developing Turdus spp. young (Grim et al. 2011). Similarly, parasitism does not significantly reduce nesting success of rufous-bellied thrushes, and parasitic shiny cowbird chicks fare poorly in these nests (Lichtenstein 2001). Shiny cowbird chicks likewise have no significant detrimental effect on creamy-bellied thrush survivorship in parasitized broods (Astie & Reboreda 2006). Lichtenstein (2001) indicated that chick-chick competition, and differences among
chicks in size were not sufficient in explaining differences in feeding rates for host versus parasitic chicks. Instead, Lichtenstein (2001) found that rufous-bellied thrush foster parents preferentially feed their own young over parasitic young.

Parental discrimination can also take the form of active removal of parasitic chicks from the nest. As we never observed dead parasitic chicks in the nest, we cannot rule out active discrimination and removal of live parasitic chicks by host parents in robins. The rejection through active removal or abandonment of parasitic chicks is increasingly documented in a wide range of hosts of interspecific brood parasites (Grim 2011), including mangrove gerygones (Gerygone laevigaster, Sato et al. 2010; Tokue & Ueda 2010), superb fairy-wrens (Malurus cyaneus, Langmore et al. 2003), skylarks (Alauda ardensis, Hegemann & Voesten 2011), and reed warblers (Acrocephalus scirpaceus, Grim et al. 2003), and in the intraspecific parasite, the American coot (Fulica americana, Shizuka & Lyon 2010). To our knowledge, similar chick discrimination has not yet been shown in any of the diverse hosts of the brown-headed cowbird. Further study should focus on identifying the mechanism of low cowbird chick survival in this system, specifically on parsing effects of chick discrimination, competition, and imperfect adaptation to a particular host, by addressing predictions associated with risk of parasitism (Schuetz 2005).

Our results reveal significantly lower proportions of host chicks fledging from eggs hatched between manipulated versus unmanipulated nests. As this survival metric specifically addresses interference at the chick stage (rather than hatching interference), this may represent handling stress on the hosts’ own chicks in manipulated nests. Alternatively, this could reflect parental bias toward feeding any cross-fostered nestling
more relative to their own chicks (but see Boncoraglio et al. 2009), or increased begging intensity for non-siblings within a nest (Briskie et al. 1994). As unmanipulated nests contained no cross-fostered chick, we are unable to test these alternatives, but future study should compare provisioning between host and parasitic chicks in order to test for decreased provisioning of own host chicks.

Our experimental results suggest that there is no specific cost of cowbird parasitism for robins, however, sharing a nest with any parasitic chick, whether cowbird or robin, imposes cost on host robins specifically at the nestling stage. Paradoxically, cowbird chicks survive more poorly when reared alongside larger and rapidly developing robin hosts. That having a parasitic chick in the nest reduces the hatching-to-fledging success of host nestmates supports predictions of a recoverable cost of cowbird parasitism for robins, which can be eliminated by ejecting the foreign egg prior to hatching. These results call for more research into brood parasite adaptations and host responses, and lays important groundwork for testing costs of parasitism for generalist brood parasites and egg-rejecting hosts.

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CHAPTER 4

High repeatability of egg rejection in response to experimental brood parasitism in the American robin (*Turdus migratorius*)

R. Croston¹, M. E. Hauber¹,²

¹ Ecology, Evolutionary Biology, and Behaviour Subprogram in Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10016, USA

² Department of Psychology, Hunter College and The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10065, USA

* Corresponding author: RCroston@gc.cuny.edu, Hauber Lab, Dept. Psychology, Hunter College, 695 Park Avenue, New York, NY 10065, USA, +1 (212)-396-6445.

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Short title
High repeatability of egg rejection in robins

Summary
Repeatability is a measure of the amount of variation in a phenotype that is attributable to differences between individuals. This concept is important for any study of behaviour, as all traits of evolutionary interest must be repeatable in order to respond to selection. We investigated the repeatability of behavioural responses to experimental brood parasitism in American robins, a robust (100%) rejecter of parasitic brown-headed cowbird eggs. Because tests of repeatability require variation between individuals, we parasitized the same robin nests twice successively with model eggs dyed with colours known to elicit rejection at intermediate rates (58-70%). We calculated the repeatability of responses to parasitism, and used a generalized linear mixed model to also test for potentially confounding effects of Julian date, presentation order, and clutch size. We found that repeatability in response to brood parasitism in this host species is high, and the best model predicting responses to sequential artificial parasitism includes only nest identity. This result is consistent with a critical assumption about egg rejection in this cowbird host as an evolved adaptation in response to brood parasitism.

Keywords: American robin, brood parasitism, brown-headed cowbird, repeatability
Introduction

Repeatability of a given behaviour is the fraction of variation in the behaviour that is attributable to variation between individuals (Falconer, 1981). This measure is useful in evolutionary biology because only phenotypes that are consistent within individuals, but variable between individuals, can respond to selection (Bell et al., 2009). Additionally, any study of behaviour assumes that population- or species-level estimates are biologically relevant and repeatable at their respective level of inquiry; otherwise, these behaviours would be of little interest, and instead would represent noise in the system (Bell et al., 2009).

Repeatability is often interpreted as the maximum value of a behaviour’s broad- and therefore narrow-sense heritability, because the measure itself includes both environmental and genetic sources of variation, whereas heritability accounts only for genetic sources of variation (Nakagawa & Schielzeth, 2010; but see Dohm, 2002). Repeatability only accounts for consistency in the behavioural display or outcome, and not the external or internal influences on it, so by definition this measure represents the maximum influence that either genetic or environmental factors can have (as in when the other is 0; Boake, 1989). Because of this relationship, repeatability is an important measure of the efficacy of natural selection on a given behavioural trait (Bell et al., 2009).

Hosts of the obligate brood parasitic brown-headed cowbird (*Molothrus ater*, hereafter cowbird) challenge evolutionary theory because most accept parasitic eggs despite the potentially high costs of rearing unrelated young (Rothstein, 1975; Lorenzana & Sealy 1999; Hauber, 2003). In hosts of most brood parasitic birds, rejection of the
foreign egg is the most common response to costly parasitism (Davies, 2000), yet few cowbird host species remove parasitic eggs from their nest (Friedmann, 1929; Takasu, 1998). American robins (*Turdus migratorius*, hereafter robin) are one of only 26 of the cowbirds over 240 hosts to remove cowbird eggs from their nests in 100% of experimental trials in regions of sympatry with cowbirds (Friedmann, 1971; Rothstein, 1982; Briskie et al., 1992).

Our earlier work has shown that robins respond specifically to parasitism by cowbirds, despite an apparent lack of sensory tuning toward the detection of cowbird eggs (Croston & Hauber *in press A*). In turn, rejecting cowbird eggs recovers the cost of parasitism paid by hatching and raising foreign young in the nest (Croston & Hauber *in press B*). These lines of evidence imply selection on robins for behavioural defenses against cowbird parasitism. Theoretical models of brood-parasite/host arms races often assume that selection on hosts results in individual hosts which are consistent in their response to parasitism, either accepting or rejecting any parasitic eggs in the nest within static ecological and perceptual contexts (Takasu, 1998; Servedio & Lande, 2003; Servedio & Hauber, 2006). Violation of this assumption, then, would call for the reassessment of previous analyses pertaining to adaptive responses to brood parasitism, as well as the heritability of egg rejection behaviours in general.

Experiments on intermediate rejecter hosts of brood parasitic eggs (defined with rejection rates ranging from 40-80%: Røskaft et al., 2002) provide the most relevant system for addressing consistency in response to parasitism and repeatability (Samaš et al., 2011). The statistical measure of repeatability requires both within- and between-individual variation in behaviour, therefore works focusing on overwhelmingly rejecter
or acceptor species (Hauber et al., 2004; Hoover et al., 2006; Honza et al., 2007) are lacking in variation necessary to demonstrate repeatability. In other words, in species with invariable responses to parasitism, consistency of response may be high, yet it can be assumed *a priori* that repeatability would be low (Bell et al., 2009; Nakagawa & Schielzeth, 2010). Based on this argument, robins should be poor subjects for studies on the repeatability of foreign egg rejection, because cowbird eggs are nearly always rejected from robin nests (Briskie et al., 1992). Thus, in order to test assumptions of repeatability in a ‘strong rejecter’ species, we set out to generate a quantitatively meaningful measurement of repeatability by evaluating responses to experimental model parasitic eggs, dyed with colours that are rejected at intermediate rates that are also statistically similar to each other. Specifically, we used yellow, red, and blue model egg colours, rejected at intermediate rates ranging from 58-70% (Croston & Hauber *in press A*; see Materials and methods), despite robust rejection (100%) of model eggs dyed to resemble parasitic cowbird background colors, and full acceptance (0% rejection) of model eggs dyed to resemble conspecific, robin eggs (Croston & Hauber *in press A*). We note, however, that any non-zero variation in behaviour is meaningful in the context of repeatability (Samaš et al., 2011, Trnka et al., 2013). It is widely known that rejection rates of different egg morphs trigger variable responses in potential hosts of egg-mimicking brood parasites, (Welbergen et al., 2001; Honza et al., 2007; Cassey et al., 2008), including several *Turdus* spp. thrushes (Grendstad et al., 1999; Moksnes et al., 1991; Sackmann & Reboreda, 2003; Astie & Reboreda, 2005; Honza et al., 2005; Polacikova & Grim, 2010; Grim et al., 2011). Here we demonstrate that the same is true of a *Turdus* host to the brown-headed cowbird, despite the lack of egg color mimicry
among parasitic eggs. This experimental approach, then, serves in part to demonstrate that rather than a static and bimodal accepter/rejecter status (Takasu, 1998), cowbird hosts can exhibit similarly variable responses to parasitism when facing variation in the foreign eggs’ coloration.

Several ecological and temporal variables, including timing of parasitism across both the laying cycle and breeding season (Rothstein, 1990; Welbergen et al., 2001), clutch size (Lyon, 2003; Servedio & Hauber 2006), and learning due to prior experience with parasitism (Lotem et al., 1992) may affect host responses to foreign eggs in the nest, effectively altering likelihood of consistent responses to parasitism (Samaš et al., 2011; Trnka et al., 2013). While we cannot control for an individual’s prior exposure to natural cowbird parasitism, testing repeatability within one nesting attempt allows us to account for exposure to natural parasitism within the time span of our study, as nests are unlikely to be parasitized in the intervening period between presentations. Although such an approach does not test for the specific role of the nests’ visual environment (e.g., total illumination, spectral content of light: Langmore et al., 2005; Honza et al., 2011) in generating consistent outcomes between nesting sites, our approach provides numerical data about individual repeatability in response to brood parasitism when facing the relatively static environmental conditions of the same breeding attempt. Specifically, we test the hypothesis that robins’ responses to sequential artificial parasitism are repeatable within a single nesting attempt. Following Samaš et al., 2011, we also tested for possible predictive effects of several ecological variables on repeatability of parasitic egg rejection. We tested for effects of Julian date, presentation order of parasitism, and clutch
size (including experimental eggs). We predict high repeatability of host responses to parasitic eggs, irrespective of presentation order, date, and clutch size.

**Material and methods**

*Study site and nests*

We studied robins during three breeding seasons May – July 2010, 2011, and 2013, in and around Ithaca, Tompkins County, NY, USA. As robins are highly commensal with humans (Sallabanks & James, 1999), and are often found nesting in bushes or on human-made structures at woodland edges, nests were located by searching in and around residential areas and parking lots. Additional nests were located by enlisting the help of local residents through Internet advertisements and list-serves, and local signboards (following Hauber, 2003 and Wagner et al., 2013). Nests were deemed active if they contained fresh and dry lining and/or eggs, and adult robins were visible in close proximity to or at the nest.
Figure 4.1a). Representative reflectance spectra for each model egg type, as measured using a high resolution spectrometer with deuterium tungsten halogen light source and 455nm solarization-resistant shielded cable (Ocean Optics Jaz spectrometer with UV-VIS light source, Ocean Optics Inc., Dunedin, FL, USA). Photos of model eggs of each colour are shown in the key. Inset shows experimental nest with three natural robin eggs and one red model egg.

Figure 4.1b). Proportions of model eggs of each colour that were rejected in 1\textsuperscript{st} versus 2\textsuperscript{nd} presentations. Sample sizes (no. of nests) are listed within each column.

Model eggs and artificial parasitism

Artificial cowbird-sized eggs were molded from plaster-of-Paris. Egg dimensions were based on average dimensions of cowbird eggs near Ithaca, NY (21.4 x 16.4 mm, 3.03 g; Lowther, 1993). Each model egg weighed between 2.6 – 3.4g, and measured 21mm x 16mm. Eggs were painted with non-toxic acrylic paint, using one of 3 artificial egg colours (yellow, red, and dark blue; Figure 4.1a) of known, and statistically similar (see Results), intermediate rejection rate (58, 64, and 70% respectively; Croston & Hauber \textit{in press A}). By definition, “intermediate” rejection includes rates of 40 – 80% (Røskaft et al., 2002).
Upon finding an active nest containing 1 or more real robin eggs, we numbered all existing eggs with a non-toxic felt-tip pen (Sharpie™ brand, black), and artificially parasitized the nest by adding one plaster-of-Paris cowbird-sized egg. Nests were artificially parasitized as available throughout each breeding season, and we did not limit timing of parasitism to a particular stage during nesting (see further discussion below). Timing of parasitism does not affect probability of egg rejection in this system (Croston & Hauber in press A). We did not remove host eggs during this experiment, as prior work on this (Briskie et al., 1992) and other Turdus species (Moskát et al., 2003; Honza et al., 2005, 2007) revealed no effect of egg addition/removal on outcome of experimental parasitism. We monitored nests by returning to each site daily, marking additional eggs, and visually determining the status (present/absent) of the artificial egg. As robins remove parasitic eggs by grasping them in their bills and carrying them away from the nest (Rothstein, 1975), eggs were considered rejected when the model egg was absent from the nest, but the nest showed no signs of hatching, abandonment, or either partial or total predation. Eggs were considered accepted if they remained in the nest for 5 days after experimental parasitism. No nests were abandoned as a result of experimental manipulation. Each nest was parasitized twice in succession using 2 different intermediately-rejected egg colours, assigned based solely on availability. In most cases (N = 14), first and second parasitism occurred in immediate succession, such that the second parasitic egg was placed in the nest at the same time as the first was found to have been ejected or accepted. In N = 2 nests, logistical constraint (weather, transportation, etc.) prevented our return to the nest on the first day after parasitism. All rejected eggs
with known latency were found rejected on day 1 following experimental parasitism, therefore we did not analyse any effects on latency to rejection.

Nests were monitored until hatching in order to assess timing of artificial parasitism relative to laying and incubation. We recorded Julian date of parasitism, clutch size, model egg colour, and presentation order for statistical analysis (see below). As nest-age at parasitism was necessarily highly correlated with presentation order and clutch size (2nd presentations always followed 1st presentations, and for presentations taking place during laying, nests always contained more eggs at 2nd presentation), these variables acted as proxies for nest age (presentation order $r_s = -0.63, N = 32, p < 0.001$; clutch size $r_s = -0.23, N = 32, p < 0.05$), and so nest age was not itself included in the analyses.

In this study, we did not individually track each male and female at the nest, but instead examined repeatability of responses to parasitism using Nest ID as a proxy for individual female identity. In most species with female-only incubation, only females reject parasitic eggs (Rothstein 1970, 1975; Palomino et al., 1998; Amundsen et al., 2002; Soler et al., 2002; Samaš et al. 2011, but see Sealy & Neudorf, 1995). In robins, and particularly in our study population, only the female typically incubates (Martin, 1973; R. Croston pers. observation). Therefore, it is unlikely that male robins play a role in rejecting parasitic eggs, and the response at each nest can provide reliable measure of individual-level repeatability. We excluded data collected across two consecutive nesting attempts at the same sites by conservatively assuming that nest ownership was shared between any two nests located within ~10m throughout any given season.
This study was conducted on private properties with consent of the affected landowners, and was approved by the Institutional Animal Care and Use Committee of Hunter College, City University of New York (# MH 2/13-T3).

**Statistical analysis**

To assess methodological robustness, we first tested for independence of parasitic egg colour and outcome (accept/reject) using Pearson’s Chi-squared test. Egg colour and outcome were not significantly related (see Results), so for subsequent analysis we pooled data from all three model egg colours.

We tested for repeatability using two different statistical approaches, following Samaš et al. (2011). First, we used a Spearman correlation to evaluate correlation between binary outcomes (accept/reject) of first and second parasitism events. Next, we calculated both the estimate of repeatability of response to parasitism and 95% confidence interval using R package irr for one-way models (v. 0.84; Gamer et al., 2012). Repeatability is estimated as the single score intraclass correlation coefficient (ICC) for a one-way model, based on the model described in McGraw & Wong (1996) and reported with 95% confidence interval (Gamer et al., 2012).

As this approach does not allow us to test for possible confounding effects of ecological covariates, we then estimated the consistency of response using a Generalized Linear Mixed Model (GLMM; Nakagawa & Schielzeth, 2010) with Gauss-Hermite approximation. Gauss-Hermite approximation is used here in place of LaPlace approximation, as this metric is most appropriate with low sample size (N < 30), and low
variability in responses, due to high rejection rates (P. Samaš and T. Grim, pers. correspondence). Here, N = 16 nests, with 66% of experimental eggs rejected overall.

All analyses were performed in R version 2.12.1. GLMM was performed using the glmer function in lme4 package. Our full model consisted of outcome (accept/reject) as dependant variable, with Nest ID, clutch size, Julian date, and presentation order as additional possible predictor variables. Although we collected data over multiple years, we did not have specific predictions associated with differences across study year, and inclusion in the full model resulted in model overfit and yielded NAs in the data set. Given that lme4 cannot handle NAs in the data, we did not include study year in the global model. N = 6 nests were sequentially parasitized in 2010, N = 8 nests in 2011, and N = 2 nests in 2013. Of 32 total presentations, 23 were conducted during laying. Studies across 6 European thrush species have addressed potential effects of parasitism during laying vs. incubation periods for egg rejection studies conducted between- (rather than within-) individuals, and found no effect on response to parasitism (Grim et al., 2011, but see Samaš et al., 2011), therefore we do not believe that our results were confounded by difference in nest stage at the time of presentation.

We constructed candidate models using GLMM, and selected among these using Akaike Information Criterion for model selection with stepwise addition in the MuMIn package (Burnham & Anderson, 2002). Here we report AICc values, which correct for finite sample sizes and relatively large values for K. Akaike weights give the relative support for a given model compared with other models in the candidate model set (Burnham & Anderson, 2002).
Results

Across all presentations, 66% of model eggs were rejected. Of 16 nests included in the study, responses were consistent across first and second presentation at 13 nests (81%). Of the N = 3 inconsistent results, all responses switched from initial rejection to acceptance of experimental parasitism between first and second model egg presentations at the same nests. Parasitic egg colour and outcome of parasitism were statistically independent (Pearson’s $\chi^2 = 0.38$, df = 2, $p = 0.83$; Figure 4.1b). Data across the three colours were pooled in all subsequent analyses.

Our initial Spearman correlation testing for independence of first and second responses within nests revealed a significantly positive correlation between these (Spearman correlation, $r_s = 0.65$, $N = 16$, $p < 0.01$), with 95% confidence interval ranging from 0.23 - 0.87. Similarly, the intraclass correlation value (indicating repeatability) for outcome of first and second parasitism was statistically positive (ICC = 0.605, with 95% CI 0.19 – 0.84).

GLMM with subsequent AIC model selection revealed that none of the ecological correlates assessed in this study are included in the best fit model predicting consistent responses to parasitism (see Table 4.1 for candidate model statistics and parameter estimates). Rather, the model best predicting outcome in our experiments includes only Nest ID as predictor.
Table 4.1. Summary of model parameters for the top five candidate models, based on AIC model selection. The best fit model is listed first, with the lowest value of AICc and the highest AIC weight ($w_I$). For fixed effects, the estimate and (S.E.) are shown.

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<th>df</th>
<th>AICc</th>
<th>ΔI</th>
<th>$w_I$</th>
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<td>2.32</td>
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Discussion

Our experiment revealed highly consistent behavioural responses to experimental parasitism in American robins. We detected the same outcome (rejection or acceptance) in response to repeated parasitism at 81% of experimental nests parasitized with intermediately-rejected model eggs. As predicted, our repeatability estimates from both Spearman correlation and ICC calculation were positive and high, indicating that within our study population, host responses to parasitism are largely consistent, yet there exists variation between individual hosts. This supports the statistical finding of high repeatability. Our best fit model predicting the outcome of experimental parasitism includes only Nest ID, and does not include any of the ecological covariates measured in this study. This indicates that these variables add no predictive value to the model, and do not affect repeatability in response to sequential experimental brood parasitism.

As high intra-individual repeatability allows for selection to act on the frequency of this behaviour within a given population (Shaw & Hauber, 2009, 2012) that repeatability in this system is high has important implications for studies of the evolution
of responses to brood parasitism. Notably, however, several hypotheses relevant to the evolutionary interactions of brood parasites and their hosts predict high repeatability in responses to parasitism (Hauber et al. *in press*). For this reason, we cannot make specific claims about the implication of high repeatability for the evolution of, for example, egg rejection by American robins in response to conspecific versus heterospecific parasitism.

An additional implication of this study was that whereas certain hosts are generally regarded as static rejecters or accepters of cowbird parasitism (Rothstein, 1990; Takasu, 1998; Winfree, 1999), it is possible to manipulate responses to parasitism in even a robust egg rejecter species by altering the colour of the model egg stimulus (de la Colina et al., 2012). Repeatability of egg rejection, as responses to parasitism themselves, is highly context- and cue-dependent. As such, the experimental approach used here may provide grounds for re-evaluating the utility of the classification of ‘acceptor’ or ‘rejecter’ cowbird hosts, and allowing flexibility in the definition according to the types (e.g., colors, shapes, etc.; Underwood & Sealy, 2006; Ban et al., 2013) of parasitic eggs accepted or rejected and the range of variation in responses that is possible (e.g. egg ejection, nest desertion. Servedio & Hauber, 2006; Hauber et al. *in press*) given a different set of parasitism cues. These types of studies can provide meaningful insight not only into mechanistic drivers of egg rejection, but the range of correlated behaviours that can arise in response to experimental parasitism, and their degree of plasticity.

In many *Turdus* thrushes, egg rejection decisions are known to depend on the degree of background colour contrast between host and parasitic eggs (Cassey et al., 2008; Croston & Hauber *in press*). As such, an unavoidable confound in studies of repeatability in nests where host eggs are otherwise unmanipulated is that the amount of
colour contrast is dependent in part on the degree of colour variation among the hosts’ own eggs within a clutch (e.g. Stokke et al., 1999; Soler et al., 2000). As multiple egg colours were used here, with varying degrees of contrast, and yet we found no significant effect of varying parasitic egg colour on outcome of parasitism, irrespective of presentation, it is unlikely that the relatively minimal colour variation within a natural clutch significantly influenced our results. In addition, nests were parasitized by the addition of a model egg without the removal of a host egg, therefore the amount of variation among host eggs remained constant across presentations, except in cases where nests were parasitized during laying.

Another potential confound of studies addressing repeatability in responses to parasitism is that responses of one individual in a pair may be pre-empted by the other individual, such that we cannot be certain that all eggs were rejected by the same individual in a pair, across experimental trials. It is rather unlikely that males play a role in parasitic egg rejection in this system (Rothstein 1970, 1975; Palomino et al. 1998; Amundsen et al., 2002; Soler et al., 2002; Samaš et al. 2011; but see Sealy & Neudorf, 1995), but further study should confirm identity of rejecter individuals using video surveillance of egg rejection by the individual member(s) of the nesting pair.

Repeatability does not always reflect heritability, such as when a behavior has been learned prior to any behavioral testing. Therefore, it remains possible that repeatability here does not lend insight into the evolvability of egg rejection itself, but may instead reflect, for example, the ability to learn to reject eggs, or unaccounted for differences in nest microclimate selection. Future study should revisit these questions under the reaction norm approach (Schlichting & Pigliucci, 1998), which describes the
range of phenotypes that can be produced given a particular genotype exposed to a range of different environmental conditions, and therefore allows for the interaction of genes and experiential factors across individual ontogeny.

It remains possible that ecological covariates not measured and included in this study accounted for some factor relevant to whether or not an individual will respond consistently to parasitism. Repeatability estimates reflect consistency both through time and across unmeasured situations (Martin & Reale, 2008), because the specific environmental situations in which a behaviour is measured in the wild cannot be entirely known. Because of this, using a framework similar to the repeatability framework to assess evidence for behavioural correlations across contexts may provide valuable further insight (Bell et al., 2009), specifically into the relative prevalence of behavioural syndromes versus behavioural plasticity, and the extent to which each exists in brood parasite-host interactions (Aviles & Parejo, 2011). Further studies should investigate the types of factors which may influence repeatability across a wide variety of behavioural contexts, and what generalizations can be made about factors influencing repeatability (see also Carter et al., 2013).

Finally, our sample sizes here were relatively small, and so it may be that we were statistically unable to detect differences in the rejection rates of the three model egg colours used in this study, confounding our results. If robins do, in fact, respond differentially to these three model egg types, our experiment could not truly test for repeatability in response to the same experimental stimulus, as this would indicate that there is not functional similarity across stimuli. Despite this limitation, our main conclusion is based on detecting highly repeatable responses to artificial parasitism.
Overall, the results indicate that egg rejection responses to foreign eggs are highly repeatable within our American robin study population, confirming a critical assumption about such responses evolving in response to selection pressure by brood parasitism. Further studies should investigate the extent to which repeatability is sustained versus decays between breeding attempts and breeding seasons, as well as the environmental-context-dependent differences in repeatability, by varying ecological contexts, physical parameters of illumination, and the properties of the parasitic stimulus itself.

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References


CHAPTER 5

The effects of clutch color variation in an egg-rejecting host of the brood parasitic brown-headed cowbird (*Molothrus ater*)

R. Croston\(^1\), M. E. Hauber\(^{1,2}\)

\(^1\) Ecology, Evolutionary Biology, and Behaviour Subprogram in Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10016, USA

\(^2\) Department of Psychology, Hunter College and The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, 10065, USA

* Corresponding author: RCroston@gc.cuny.edu, Hauber Lab, Dept. Psychology, Hunter College, 695 Park Avenue, New York, NY 10065, USA, +1 (212)-396-6445.
ABSTRACT

An evolutionary history with brood parasites can reciprocally affect hosts’ egg coloration, as the amount of variation within a clutch can either facilitate or constrain the recognition and rejection of parasitic eggs. Where parasitic eggs mimic hosts eggs, color variation within clutches may 1) differ from color variation between clutches, or 2) impair hosts’ ability to recognize and reject parasitic eggs, depending on the cognitive mechanism(s) used for egg recognition. Because of this, hosts of mimetic brood parasite are predicted to have significantly less variation in egg color within a single clutch than between clutches. Similarly, in response to experimental increase in egg color variation, rejection rates for parasitic eggs in the nests of these hosts are predicted to decrease. For hosts of non-mimetic parasites, however, intraclutch egg color variation is not constrained by brood parasitism, therefore the ability to reject parasitic eggs should not change in response to manipulation of color variation within clutches. Here we tested predictions associated with parasitism by a non-mimetic brood parasite, the brown-headed cowbird, in a robust egg-rejecting host, the American robin, versus the alternative hypothesis that robin egg color variation reflects a history of parasitism by conspecific, functionally mimetic parasites. We quantified differences within and between clutches as perceived by hosts using spectrophotometric measures and avian visual modeling. We also manipulated intraclutch color variation, measuring responses to simulated cowbird parasitism in nests where color variation was artificially enhanced or constrained. We found that egg color variation is greater between than within robin clutches, and experimental manipulation of color variation does not affect rejection rates. Our results support egg rejection as an evolutionary response to both mimetic and non-mimetic brood parasites. We discuss the
further need for studies addressing these hypotheses for hosts on non-mimetic brood parasites, and the potential role of cognitive mechanisms in predicting differential responses to parasitism among these hosts.
INTRODUCTION

Hosts of brood parasitic birds face fitness costs associated with the rearing of genetically unrelated parasitic offspring (Davies 2000). Many hosts, across widely divergent brood parasite-host systems, have evolved defenses which either decrease the chances of being parasitized, or reduce the costs incurred as a result of parasitism (Rothstein 1975; Davies 2011). These defenses range from aggressive responses to adult brood parasites (Sealy et al. 1998; Feeney et al. 2012) through egg rejection (Mosknes et al. 1991; Langmore et al. 2005) to the rejection of brood parasitic chicks (reviewed in Grim 2011).

The recognition and removal of parasitic eggs from the nest is the most common host defense against parasitism (Rothstein 1990, Grim 2011). Egg rejection, however, is an imperfect defense, and can itself incur costs for hosts through the loss of an abandoned clutch, misrecognition, or accidental damage to the hosts own eggs (Lorenzana and Sealy 2001; Antonov et al. 2006). Because of these costs, evolutionary theory predicts that hosts involved in an arms race with brood parasites will be under repeated selective pressure to avoid recognition errors (Moskát and Hauber 2007). For some brood parasites, this results in selective pressure to lay eggs mimicking host eggs in appearance (mimetic eggs), while others lay eggs that do not appear to mimic those of their hosts (non-mimetic eggs, Davies and Brooke 1988).

For hosts of mimetic brood parasites, there are at least two strategies toward reducing the likelihood of recognition errors. Individuals of a parasitized species can evolve towards 1) reduced intraclutch color variability, and/or 2) egg appearance unlike the parasitic eggs, effectively increasing interclutch variability (Davies and Brooke 1989,
One or both of these clutch variation patterns has been observed in many host species of the common cuckoo (*Cuculus canorus*, Øien et al. 1995; Soler and Møller 1996; Stokke et al. 2002; Moskát et al. 2002; Avilés and Møller 2003; but see Karcza et al. 2003) and diederik cuckoo (*Chrysococcyx caprius*, Lahti 2005), as well as in rejecters of intraspecific (functionally mimetic) parasitic eggs (Stokke et al. 1999). Many other studies have, however, found no support for these patterns (Lotem et al. 1995; Stokke et al. 2002; Procházka and Honza 2003; Avilés et al. 2004; Lovaszi and Moskát 2004; Cherry et al. 2007), and the literature as a whole is equivocal as to what extent brood parasitism and egg recognition fuel or limit evolutionary trajectories of variation in both intra- and intraclutch egg color variability (Table 5.1).

Hypotheses pertaining to the effects of parasitism on intraclutch egg color variation, and effects of this variation on rejection rates, have gone largely untested in hosts of non-mimetic parasites, as there is little intraspecific variation in response to parasitism for these hosts (Peer et al. 2010). In the only known study to date on the relationship between rejection rate and degree of intraclutch variation in a brown-headed cowbird (*Molothrus ater*) host, Peer et al. (2010) found that cowbird egg rejection is more likely when intraclutch variation is lower for common grackles (*Quiscalus quiscula*, see Table 5.1). In our earlier studies, we have found that one robust egg-rejecting cowbird host, the American robin (*Turdus migratorius*) rejects cowbird eggs more frequently than predicted based on a best-fit model describing rejection rates of cowbird eggs (Croston and Hauber 2013). Likewise, parasitism exerts selective pressure on robins to avoid rearing their young alongside cowbirds, however, the cowbirds chicks
themselves also fare poorly when reared alongside robin young (Croston and Hauber in press. Because cowbird chicks survive poorly in robin nests, selective pressure toward egg rejection may occur not as a direct evolutionary consequence of cowbird parasitism, but rather as a byproduct of adaptive removal of the eggs of conspecific, functionally mimetic parasites.

Conspecific parasitism is a necessary precursor to interspecific parasitism (Yamauchi 1995), therefore, defenses employed against interspecific parasites may have evolved in defense of conspecific parasitism. Conspecific parasitism is uncommon among Turdus thrushes, but is known to occur in American robins at our study site (Gowaty and Davies 1986). Defenses against conspecific parasitism are similarly uncommon, likely due to relatively low cost of parasitism and difficulty identifying parasitic eggs and young (Rothstein 1990). American robins are robust rejecters of interspecific parasitic eggs, but do not reject those of conspecifics (Briskie et al. 1993).

Across existing studies either 1) investigating patterns of egg rejection responses to within-clutch egg color manipulation and 2) measuring observable differences in egg color variation within versus between host clutches in hosts of mimetic brood parasites, there is considerable variation in adherence to predictions stemming from the hypothesis that intra clutch egg color variation is a mechanism used by hosts in the recognition of foreign eggs (Table 5.1). With an increasing difference in appearance between host and parasitic eggs (i.e. poorer egg mimicry), the relative importance of inter- and intraclutch egg color variation in host egg color in determining the relative fitness of the rejecter strategy decreases (Stokke et al. 2007). Therefore, when parasitic eggs are non-mimetic, as a cowbird egg, parasites inflict no selective pressure on host egg color variability.
within and/or between clutches (Stokke et al. 2002), and no difference is predicted between within-clutch versus between-clutch color variation.

We combine observational and experimental approaches to analyze within and between-clutch color variation in a population of American robins, a robust egg-rejecting host of brown-headed cowbirds (Rothstein 1975; Briskie et al. 1993). In our earlier study, we have found that the cost of cowbird parasitism for robins is low (Croston and Hauber in press), yet robins are one of only ~26 hosts of the extremely generalist (Friedmann 1929, 1971) brown-headed cowbirds (Peer and Sealy 2004) to reject artificial and real cowbird eggs in 100% of trials where nests are experimentally parasitized (Rothstein 1975; Briskie et al. 1993). Here we test predictions associated with the hypotheses that robin egg rejection has evolved in response to 1) non-mimetic (as a cowbird) versus 2) mimetic (as a conspecific) parasites. We investigate the degree of variation in egg color within versus between natural robin clutches, and test for effects of manipulating egg color variation within clutches on the likelihood of parasitic egg rejection. If egg rejection in robins evolved in response to parasitism by non-mimetic cowbirds, we predict no difference in degree of egg color variation within or between clutches. Likewise, we predict no effect of artificially altering color variation within clutches on the ability of hosts to discriminate and reject parasitic eggs. If, however, egg rejection in robins evolved in response to parasitism by conspecifics, we predict that egg color variation among clutches will exceed variation within clutches, and artificially increasing and decreasing egg color variation within clutches will effect the rate of rejection for artificial parasitic eggs.
Table 5.1a
Summary of published studies on egg rejection responses to brood parasitism, where the methodology included the experimental increase of intraclutch color variation. “Parasite Mim./Non.” indicates whether natural parasitic eggs mimic those of hosts. “Exp. Mim./Non.” indicates whether eggs used in artificial parasitism mimicked those of hosts. “Effect” indicates the induced change in the rate of rejection of experimental eggs.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Parasite Mim./Non.</th>
<th>Exp. Mim./Non.</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Mim.</td>
<td>Mim.</td>
<td>None</td>
<td>Karcza et al. 2003</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Mim.</td>
<td>Mim.</td>
<td>Negative</td>
<td>Moskát et al. 2008</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Mim.</td>
<td>Both</td>
<td>Negative</td>
<td>Ban et al. 2013 *</td>
</tr>
<tr>
<td>Anomalospiza imberbis</td>
<td>Prinia subflava</td>
<td>Mim.</td>
<td>Mim.</td>
<td>Negative</td>
<td>Stevens et al. 2013</td>
</tr>
</tbody>
</table>

Table 5.1b
Summary of published studies of observational tests of relationship between intraclutch egg color variability and rejection rate. Parasite Mim./Non.” indicates whether natural parasitic eggs mimic those of hosts. “Correlation” indicates the direction of correlation (if any) between color variation within (“Correlation intra-”) and between (“Correlation inter-”) and the rejection rate of parasitic eggs.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Parasite Mim./Non.</th>
<th>Correlation intra-</th>
<th>Correlation inter-</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuculus canorus</td>
<td>Various</td>
<td>Mim.</td>
<td>None</td>
<td>Positive</td>
<td>Øien et al. 1995</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Various</td>
<td>Mim.</td>
<td>Negative</td>
<td>Positive</td>
<td>Soler and Møller 1996</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Mim.</td>
<td>Positive</td>
<td>NA</td>
<td>Lotem et al. 1995</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Mim.</td>
<td>None</td>
<td>Positive</td>
<td>Moskat et al. 2002 **</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Acrocephalus arundinaceus</td>
<td>Nonmim.</td>
<td>Negative</td>
<td>NA</td>
<td>Cherry et al. 2007</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Sylvia communis</td>
<td>Nonmim.</td>
<td>None</td>
<td>NA</td>
<td>Stokke et al. 1999</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Anthus pratensis</td>
<td>Mim.</td>
<td>Negative</td>
<td>NA</td>
<td>Procházka and Honza 2003</td>
</tr>
<tr>
<td>Cuculus canorus</td>
<td>Lanius collario</td>
<td>Mim.</td>
<td>None</td>
<td>NA</td>
<td>Avilés and Moller 2003 **</td>
</tr>
<tr>
<td>Clamator glandarius</td>
<td>Pica pica</td>
<td>Mim.</td>
<td>Negative</td>
<td>NA</td>
<td>Lovászi and Moskát 2004</td>
</tr>
<tr>
<td>Clamator glandarius</td>
<td>Pica pica</td>
<td>Mim.</td>
<td>Positive</td>
<td>NA</td>
<td>Avilés et al. 2004</td>
</tr>
<tr>
<td>Chrysococcyx caprius</td>
<td>Plocus cucullatus</td>
<td>Mim.</td>
<td>Negative</td>
<td>Positive</td>
<td>Lahti 2005</td>
</tr>
<tr>
<td>Molothrus ater</td>
<td>Various</td>
<td>Nonmim.</td>
<td>None</td>
<td>None</td>
<td>Stokke et al. 2002</td>
</tr>
<tr>
<td>Molothrus ater</td>
<td>Quiscalus quiscula</td>
<td>Nonmim.</td>
<td>Negative</td>
<td>NA</td>
<td>Peer et al. 2010</td>
</tr>
</tbody>
</table>

* To our knowledge, this is the only previous study to experimentally both increase and decrease intraclutch color variation.
**Studies compared inter- and intrACLuch color variation between 2 populations, one in sympatry and one in allopatry with cuckoos. Positive correlation for interclutch color variation is derived from statistical difference between these two populations. Lack of correlation for intraclutch color variation is derived from lack of statistical difference between these two populations.
METHODS

Study site and nests

This study took place in and around Ithaca, Tompkins County, NY, USA from May-July in the breeding seasons 2010 - 2012. Nests were located through searching in and around human-made structures and clearing edges, especially in residential areas and farmland. Additional nests were located with the help of local residents using classified ads, signboards, and local internet listserves. All manipulations and measures took place on private property and with the explicit consent of the property owner.

Egg color measurement and avian visual modeling

During the 2012 breeding season, we quantified eggshell color for full, unmanipulated American robin clutches. All clutch color measurements were taken on either the day of, or the first day following clutch completion, as blue-green color may fade over the course of incubation (Moreno et al. 2011). We quantified egg color across the entire avian visual spectrum by measuring spectral reflectance using a high resolution spectrometer with deuterium tungsten halogen light source and 455μm solarization-resistant shielded cable (Ocean Optics Jaz portable spectrometer with UV-VIS light source, Ocean Optics Inc., Dunedin, FL, USA). Measurements were taken using a fiber optic probe held perpendicular to the egg surface for each individual measurement. The spectrometer was calibrated using a Spectralon reflectance standard (WS-1, Ocean Optics, Inc., which reflects > 95% of UV and visible light), and a black-box standard, which measures baseline noise in the spectrophotometer. The percent reflectance at each wavelength was calculated automatically with reference to the light and dark standards,
using OOIBase32 software (Ocean Optics Inc.). To minimize measurement error, each egg was measured nine times, including three measurements each at the blunt pole, middle, and sharp pole. The spectrometer was calibrated at regular intervals throughout sampling.

To estimate degree of color variation both within and between unmanipulated robin clutches with respect to the spectral sensitivities of avian photoreceptors (Bennett and Thery 2007), we used the Vorobyev and Osorio (1998) model for tetrachromatic vision in AVICOL v5 avian visual modeling software (Gomez 2010). American robins are a UVS species (Chen et al. 1984; Chen and Goldsmith 1986; Aidala et al. 2012), but detailed spectral sensitivity data are not as yet available for this species. We therefore extracted spectral sensitivity data for a congener, the European blackbird *T. merula*, from data published in Hart et al., 2000 using Vistametrix software (Vista Metrix 1.3, SkillCrest LLC, www.skillcrest.com) and ranging from 330 – 700nm. AVICOL requires sensitivity data ranging from 300 – 700 nm; we set photoreceptor absorbance for 300 – 330 nm to 0 (*sensu* Igic et al. 2010, 2012). Relative cone densities were set to UVS: 1.0, SWS: 1.78, MWS: 2.21, LWS: 1.96, and Weber fraction was set to 0.1 (Hart et al. 2000; *sensu* Igic et al. 2010). As the ability to discriminate different colors is influenced by environmental light (Vorobyev and Osorio 1998, but see Honza et al. 2011), we used published ambient light irradiance data for broken canopy forest (Vorobyev and Osorio 1998), which may most closely simulate the variable forest-edge light environments in which many American robins nest, even when breeding in sub/urban sites (Sallabanks and James 1999).
Prior to analysis, we applied a triangular smoothing correction to each egg spectra using triangular smoothing over 30 nanometers, available as a function within AVICOL, to attenuate the effect of spectrometer noise on the visual model. AVICOL extracts receptor catch quanta specific to each single-cone receptor type, and combines these with the known spectral sensitivities of the model taxon’s visual system (here *T. merula*) to quantify photoreceptor activity across the entire avian spectral sensitivity range and quantify birds’ abilities to distinguish between any two colors as the perceptual distance between spectra (ΔS) or as JNDs (‘just noticeable differences’). By definition, JNDs values greater than 1.0 indicate a chromatic difference that is discriminable based on our estimates of avian spectral sensitivities (Osorio and Vorobyev 1996). AVICOL also extracts discriminability based on achromatic contrasts using the sum of the sensitivities of MWS and LWS cones, as these are similar to the sensitivities of rods and principal double-cone cells in the avian retina (Hart et al. 2000).

For the sensory analysis, we extracted photoreceptor catches for each of the four avian single-cone receptors, and normalized these to 1 within the total reflectance of each egg, such that for each egg, we have calculated the proportion of total receptor catch that is attributable to each photoreceptor. We compared mean quantum catches for each photoreceptor across all nests using independent univariate ANOVAs, with the proportionate receptor catches (PrUVS, PrSWS, PrMWS, PrLWS) as response variables, and Nest ID as predictor. Likewise, we compared mean achromatic quantum catches among nests, repeating the above approach with achromatic quantum catch data for each egg, and comparing means across nests.
Finally, we compared discriminable difference between eggs sharing a nest and eggs not sharing a nest as JNDs. To do this, we calculated JNDs differentiating each egg from every other egg in the data set. Then, we randomly selected among these paired comparisons such that each egg was included in the analysis only once. We compared mean within-nest JNDs to mean between-nest JNDs using univariate ANOVA, with type of comparison (within nest/between nest) as a predictor and JNDs as response.

Figure 5.1 Representative spectra showing each of the three colors used in the egg rejection experiment, with natural American robin egg spectra. Pale-mimetic and vivid-mimetic paints were used to manipulate the color of real robin eggs. Blue paint was used to color plaster-of-Paris model robin eggs. The unmanipulated spectrum represents the average for real robin eggs.
Egg rejection experiment

To experimentally test whether own clutch color variation contributes to the ability of American robins to recognize and reject foreign eggs, we manipulated egg color within clutches, experimentally decreasing or increasing intraclutch color variability (Moskát et al. 2008). We altered the eggs according to two treatments: in each, we removed eggs one at a time from nests, and painted each with one of two different blue/blue-green paints (acrylic, Artist’s Loft™), chosen by spectrophotometric specifications of hue as determined by wavelength at peak reflectance, and of known, low (0 – 20% for cowbird-sized model eggs) rejection rates. Eggs were allowed to dry fully before being returned to the nest. In order to increase the amount of color variation within a clutch (increased color variation treatment; IV), we painted two eggs with either pale robin-mimetic or vivid robin-mimetic paint (Figure 5.1) at random, and the third egg was painted the second blue-green shade (Figure 5.1; see also inset). The second treatment group consisted of nests where the amount of color variation within a clutch was artificially decreased by painting all 3 eggs in the clutch with the same paint shade (Vivid robin-mimetic paint, decreased color variation treatment; DV). We added a third, unmanipulated group (UNM) of nests using data from previous years (Moskát et al. 2010). These nests consisted of clutches containing 2 - 4 eggs whose colors were not altered but where the nest was treated otherwise identical to IV and DV nests, and inspected with the same frequency and manner.

Subsequent to manipulating host egg color, we returned the following day, and artificially parasitized nests with plaster-of-Paris model eggs painted a third shade of blue (Figure 5.1), also of known rejection rate (58%) from previous experiments. We chose a
blue model parasitic egg because responses to model mimetic cowbird eggs are invariable in our robin population, whereas exposure to the blue model yields variable host response. Model ‘parasitic’ eggs mimicked the mass and dimensions of real cowbird eggs, measuring 2.6 – 3.3 g and with dimensions 21 x 16 mm (Bent 1958, as cited in Lowther 1993). No significant bias has been attributable to the use of model eggs in artificial parasitism studies with American robins (Rothstein 1975; Hill and Sealy 1994). We employed a one-day latency between manipulation and experimental parasitism in order to ensure that no host eggs were rejected as a result of color manipulation (pale mimetic host egg was rejected at N = 1 nest, out of N = 27 total nests). Where a 4th egg was laid after manipulation, this was removed in order to keep clutch sizes consistent across IV and DV nests. In most cases, hosts were theoretically able to view these 4th eggs alongside painted eggs for a period of 1 – 4 hours before removal. Because the degree of variation remained greater in IV than in DV treatments even in nests where a 4th egg was laid, and because hosts were free to view their full unmanipulated clutches in cases where we discovered a clutch already containing 3 eggs, we do not consider that this limited exposure to unmanipulated eggs has effected the robins’ rejection decisions (Moskát et al. 2010).

We monitored experimental and control nests by returning daily and visually determining the status of the artificial egg, using binoculars and small nest-mirrors as necessary. Eggs were considered rejected if they were not present in the nest on the day following manipulation, except when hatching or predation may have occurred. Model eggs were considered accepted if they remained in the nest for 6 consecutive days (sensu Honza et al. 2007), after which nests were emptied, as painting the egg shells inhibits
respiratory exchange and thereby prevents proper embryonic development. By disposing of the eggs immediately, we minimize the loss of parental investment and maximize likelihood of renesting. For each nest we recorded the treatment, date of parasitism, nesting stage (laying/incubation), and outcome of parasitism (accept/reject).

Frequency tables of treatment (IV, DV, control) and outcome (accept/reject) data were analyzed using Fisher’s exact test with Monte Carlo simulation based on 2000 replicates. We next evaluated possible effects of nesting stage, clutch size, and Julian date by including these as covariates in fitting a binomial logistic generalized linear mixed model (GLMM) with treatment group and incubation stage as additional possible predictors, and year as a random variable. Experimental parasitism during the laying stage was defined as taking place at any time before or on the day the last egg was laid; at any point beyond it was considered as taking place during the incubation stage.

Because our hypotheses predict no effect of manipulating clutch color variation, we have also included here a power analysis for our experimental manipulation. All analyses were conducted in R version 2.12.1.

RESULTS

*Avian visual modeling of egg color analysis*

Mean quantum receptor catches for natural robin eggs differed significantly more between nests (N = 23) than expected based on variation within nests, for four avian single-cone photoreceptors (UVS, SWS, MWS, LWS), and for achromatic photoreceptors (Table 5.3). Mean chromatic discriminability, as JNDs, (N = 35
comparisons) was greater between nests than would be expected based on variation
within nests. Data and test statistics from avian visual modeling are summarized in Table
5.2.

**Egg rejection experiment**

We found no significant effect of experimental increase or decrease in intraclutch
color variation on probability of egg rejection (across all groups Fisher’s exact test, \( p =
0.59 \); with Monte Carlo simulation, \( p = 0.60 \); Figure 5.2). Likewise, the probability of egg
rejection does not differ between IV and DV nests (Fisher’s exact test, \( p = 1 \); with Monte
Carlo simulation, \( p = 1 \)). Likewise, likelihood of egg rejection was not significantly
predicted by treatment, clutch size, Julian date of artificial parasitism, or incubation stage
(binomial logistic regression; see Table 5.3).

We conducted a power analysis using the true effect size from the Fisher’s exact
test above, as Cramer’s \( V \). Based on Cramer’s \( V = 0.16 \) for our actual data set, statistical
temperature \( = 0.10 \). To achieve statistical power of 0.8 for this low true effect size, \( N = 396
manipulations would be necessary.
Table 5.2
Univariate ANOVA outputs for the differences in the proportional photoreceptor catches between eggs within versus between unmanipulated host nests. For each photoreceptor type, ‘Mean (SE)’ represents the proportionate receptor catch per egg, and standard error. JNDs values indicate discriminable chromatic difference between two eggs, as perceived by avian visual physiology (see Methods). For JNDs, mean JND values are shown both for within (W) and between (B) nest comparisons. Significant p values for JNDs indicate that mean discriminability was greater between nests than would be expected based on variation within nests. For all measures, there is significantly more variation between nests than within clutches.

<table>
<thead>
<tr>
<th>Photoreceptor</th>
<th>Mean prop. catch/egg (SE)</th>
<th>Num. df</th>
<th>Den. df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVS</td>
<td>0.03(0.00)</td>
<td>19.00</td>
<td>14.45</td>
<td>6.25</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>SWS</td>
<td>0.22(0.00)</td>
<td>19.00</td>
<td>14.17</td>
<td>10.28</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>MWS</td>
<td>0.37(0.00)</td>
<td>19.00</td>
<td>14.38</td>
<td>8.86</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>LWS</td>
<td>0.38(0.00)</td>
<td>19.00</td>
<td>15.50</td>
<td>72.08</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Achrom</td>
<td>20.78(0.55)</td>
<td>19.00</td>
<td>14.82</td>
<td>10.12</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>JNDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.89(0.53)</td>
<td>1.0</td>
<td>19.98</td>
<td>5.86</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>B</td>
<td>2.26(0.19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3
Summary of binomial GLMM outputs describing the effects of experimental manipulation, nesting stage (laying versus incubation), and Julian date on the likelihood of the rejection of ‘parasitic’ eggs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Error</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (IV)</td>
<td>-0.15</td>
<td>1.00</td>
<td>-0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>Treatment (Con)</td>
<td>0.92</td>
<td>0.86</td>
<td>1.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Nesting stage</td>
<td>0.01</td>
<td>0.81</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Julian date</td>
<td>0.01</td>
<td>0.02</td>
<td>0.45</td>
<td>0.65</td>
</tr>
<tr>
<td>Clutch size</td>
<td>-0.31</td>
<td>0.60</td>
<td>-0.51</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Figure 5.2 Summary of results of experimental parasitism following manipulation of clutch contents. Bars represent rejection rates for parasitic eggs in each experimental group. Error bars represent standard error around the mean. In total, data from 57 experimentally parasitized nests were included in this study (IV nests, N = 12; DV nests, N = 8; unmanipulated nests, N = 28). There were no significant differences among treatment groups.
DISCUSSION

In accordance with our predictions stemming from the hypothesis that evolution of egg rejection in robins is an adaptive response to cowbird parasitism, the results of our observational measures of unmanipulated clutch color variation reveal significantly higher perceivable variation between clutches than within clutches, across the sensitivity ranges for all four avian photoreceptors. However, our behavioral experiment has shown that the degree of color variation within host clutches had no effect on hosts’ ability to recognize and reject parasitic eggs, and rejection of our model parasitic eggs was independent of intraclutch color variation. Based on our predictions for hosts of mimetic versus non-mimetic brood parasites, our results here provide evidence in support of both adaptive scenarios.

These analyses have revealed a standing issue in testing hypotheses of egg color variation for hosts of non-mimetic brood parasites. Under the non-mimetic brood parasite scenario, we predict no effect of parasitism on either egg color variation within a clutch, or responses to experimental manipulation of color variation within a clutch. Because our predictions are negative, and do not differ across observational and experimental studies, caution must be exercised in the interpretation of such data, and findings are of limited usefulness in identifying general patterns of host-brood parasite evolution. Indeed, even for hosts of mimetic brood parasites, there is considerable variation in adherence to predictions based on host-parasite coevolution (Table 5.1). These differences may be the result of different cognitive mechanisms employed by hosts in the decision to reject parasitic eggs.
Predictions about the effects of parasitism on egg color variation may differ depending on the cognitive mechanism that hosts use to recognize parasitic eggs (summarized in Table 5.4), and this may partially explain differences in the relationship between degree of variation within and between clutches and the rate of rejection of foreign eggs across hosts of mimetic brood parasites (Table 5.1). Hosts can recognize parasitic eggs using one or more of the following cognitive mechanisms: in 1) discordancy-based recognition, hosts use the current nest contents to assess egg identity, and remove egg(s) which are unlike the rest of the clutch (Rothstein 1974; Lyon 2007). In 2) template-based recognition, host females compare clutch contents to a template of their own eggs from memory, with each egg evaluated against the acceptance threshold anchored by this template (Lotem et al. 1995; Hauber et al. 2006). The template may be either learned, such that hosts imprint on their own eggs by examining the first laid egg or several of their earlier laid eggs, or it may be inherited (Hauber et al. 2004; Hoover et al. 2006; Moskát and Hauber 2007); template based recognition also allows the recognition and rejection of foreign eggs when no host eggs are available in the clutch due to multiple parasitism (Ban et al. 2013). Finally, in 3) online self-referent phenotype matching, hosts use the current nest contents to assess egg identity, but rejection is not dependent on relative numbers of each egg type within the clutch, as each egg is compared with the hosts known eggs (Ban et al. 2013, *sensu* Hauber and Sherman 2001) as identified shortly after laying (Hauber and Sherman 2001; Moskát and Hauber 2007).

Notably, parasitism by a non-mimetic parasite is predicted to have no direct effect on the intraclutch color variation or rejection rates for hosts, therefore, for hosts of non-mimetic parasites, predictions do not change depending on host cognitive mechanism –
parasitism involving a sufficiently distinct foreign egg phenotype will have no effect on host clutch color variation under any cognitive scenario. Because of this, investigations of intraclutch color variation and its effect on egg rejection are of limited utility in parsing the cognitive mechanisms which may underlie egg rejection in these hosts and confound effects of parasitism by a mimetic versus non-mimetic parasite on egg color variation and rejection rates. If hosts of non-mimetic parasites utilize a discordancy-based recognition system, experimental manipulation of intraclutch variation cannot effect rejection unless clutch contents are modified specifically to make hosts eggs appear similar to parasite eggs, guaranteeing that these eggs are generalizable and recognizable as foreign and allowing the test to focus only on responses elicited by differences in egg number. Likewise, if hosts utilize template-based recognition, the characteristics of the existing clutch are not relevant to decision-making, irrespective of variation, unless the recognition template is updated frequently and/or parasitism rates are consistently high. If hosts utilize online self-referent phenotype matching, experimental manipulation of intraclutch color variation can only affect rejection rates if hosts are now allowed to view their own eggs at any point prior to manipulation.

Similarly, we have predicted above that when parasites inflict no selective pressure on host egg color variation (as with a non-mimetic parasite), there is no difference in variability within versus between host clutches. However, where egg color is not under selective pressure from brood parasitism, we should still assume a priori that egg color varies less within than between nests, as in this scenario egg color is free to respond to any of a wide range of physiological, genetic, and environmental factors. (Weidinger 2001; Moreno and Osorno 2003; Avilés et al. 2007; Lovell et al. 2013).
Therefore, predictions for egg color variation for hosts of non-mimetic parasites, under each cognitive mechanism are identical to those for hosts of mimetic parasites, and mechanisms underlying this pattern of variation remain obscured.

Importantly, we here tested for effects of both increasing and decreasing clutch color variation. To our knowledge, the effect of decreasing clutch color variation has been addressed in a single study, Ban et al. (2013), in which investigators manipulated entire great reed warbler clutches, dying entire clutches with the same color paint. Great reed warblers are parasitized by mimetic common cuckoos, therefore decreasing variation should result in a decrease in rejection rates. Ban et al. (2013) found, however, that while rejection rates in these nests did decrease relative to rejection rates in their single parasitism treatment, they were not statistically different from rejection rates in nests where intraclutch variation had been experimentally increased. Notably, rejection rates across different color manipulations remained proportional across their different color treatments such that blue eggs, for example, were always rejected least often and orange eggs most often. This implies that hosts use a relative color-based sensory threshold to make decisions whether or not to reject foreign eggs, but responses may be modified by context, as in the frequency of parasitism (Ban et al. 2013). Their conclusion highlights the need for further study testing for effects of both increasing and decreasing intraclutch color variation, in order to test for context-dependent effects on foreign egg rejection.
Table 5.4. Summary of predictions by different cognitive mechanisms of egg recognition for intra- and interclutch color variation as a result of coevolution with mimetic versus nonmimetic brood parasites. “Pred. effect” represents the direction of the predicted effect of experimental increase in intraclutch color variation on the probability of rejecting the parasitic egg. Note that for hosts of non-mimetic brood parasites, differences in color variation within versus between clutches are predicted to result from factors outside of selective pressure from brood parasites (see Discussion).

<table>
<thead>
<tr>
<th>Mimetic parasite</th>
<th>Discordancy</th>
<th>Template</th>
<th>Online self-reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-</td>
<td>Inter-</td>
<td>Pred. effect</td>
</tr>
<tr>
<td>Decrease</td>
<td>Increase</td>
<td>Negative</td>
<td>Decrease</td>
</tr>
<tr>
<td>Decrease</td>
<td>Increase</td>
<td>No effect</td>
<td>Decrease</td>
</tr>
</tbody>
</table>
It remains possible that egg rejection has been retained in American robins as a phylogenetically conserved behavior from egg-rejecting ancestors, and is neither an adaptive response to con- or heterospecific parasitism. True thrushes (genus *Turdus*) are among the most widely spread and speciose of songbird genera, with 65 extant species having a nearly global distribution (Voelker et al. 2009). As such, the true thrushes are sympatric with many brood parasitic species. *Turdus* species sympatric with common cuckoos are rarely parasitized (Grim et al. 2011), and there is no record of cuckoo eggs mimicking those of thrushes (Moksnes and Røskaft 1995). Yet, for example, the song thrush *T. philomelos* and European blackbird *T. merula* reject model common cuckoo at 77.8% and 75% respectively among European populations, although neither species is regularly parasitized (Polacikova and Grim 2010). Introduced New Zealand song thrush and blackbird populations reject non-mimetic model common cuckoo eggs (song thrush 65%, blackbird 60%; Hale and Briskie 2007). The same song thrush population also rejects 36% model conspecific eggs (Hale and Briskie 2007).

Only the American robin is a known robust rejector of parasitic cowbird (genus *Molothrus*) eggs, despite their non-mimetic appearance. Anti-parasite behaviors are maintained in this lineage is paradoxical in light of their low rates of parasitism. Egg rejection is, however, usually maintained in a lineage even in the absence of parasitism (Bolen et al. 2000; Peer and Sealy 2004; Grim 2006; Lahti 2006). Further study should explicitly address this hypotheses based on a phylogenetic reconstruction of egg rejection across the *Turdus* clade.

Although we manipulated egg color as soon as possible upon discovery of a nest, females had time to view their eggs prior to manipulation, therefore it is possible that the
change in egg color itself impacted responses to parasitism. Under template-based recognition or online self-referencing, this could occur through inhibiting the females ability to learn/reinforce the appearance of her own eggs (Moskát et al. 2008).

Alternatively, robin females may have already learned the appearance of their clutch prior to manipulation (Rothstein 1974). However, because we found no significant differences among rejection rates of our model parasitic egg for our IV, DV, and control treatments (Figure 5.2), exposure to eggs prior to manipulation does not appear to have impacted our results (Moskát et al. 2010).

Here, our experimental sample size was relatively low, therefore Type II error (i.e. failure to reject a false null hypothesis) remains possible. We have included a power analysis demonstrating robustness of our finding that there is no statistical difference in rejection rates across our three experimental groups. That the effect size for this manipulation is small enough (Cramer’s V = 0.16) that several hundred nests would be required in order for outcomes to appear different based on an alpha level of 0.05, demonstrates that there is no significant true effect increasing or decreasing intraclutch color variation on parasitic egg rejection rates.

Overall, our experiment has shown support for predictions associated with egg rejection in robins as an adaptive response to historical parasitism by both non-mimetic cowbirds and mimetic conspecifics as parasites. We have demonstrated that for hosts of non-mimetic parasites, there is yet need for the development of specific protocols allowing for differential predictions of egg color variation and its effects on foreign egg rejection. Parsing, and for parsing cognitive mechanisms used to make rejection decisions. Further research should be focused toward devising new ways to test
predictions associated with the effects of brood parasitism on egg color variation for hosts of non-mimetic parasites, and including tests for effects of both increasing and decreasing intraclutch color variation.
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CHAPTER 6

Prospectus

Through my doctoral research, I have attempted to tease apart the mechanisms underlying the hypothesis that egg rejection in the American robin occurs as a specific adaptation to its brood parasite, the brown-headed cowbird. These studies have painted a compelling picture regarding the shared evolutionary history of robins and cowbirds, and identified several mechanisms underlying the parasite-specific responses of this host species. Though it is impossible to test for coevolution without a broader comparative approach (Anderson et al. 2009), and while we have not here addressed any reciprocal adaptations in the cowbirds themselves, mechanistic considerations of host and brood parasite interactions are of great value to our understanding of proximate outcomes that have evolved as a result of selective pressures on host and parasite morphologies, sensory systems, and behavioral strategies, and the myriad ways in which these reciprocally influence the life histories of parasitic birds and their hosts.

In Chapter 2, I have shown that robins’ rejection of foreign eggs occurs specifically in response to cowbird parasitism. The likelihood of rejection of model eggs of artificial colors spanning the entire avian spectral sensitivity range is best predicted by a model containing input from all four photoreceptors. Likewise, overall avian-perceivable chromatic difference between natural and model eggs (JNDs) predicts rates of rejection. These results indicate that model egg colors perceived as more different from the robins own eggs will be rejected at higher rates.
In contrast, visual modeling here revealed that robin-mimetic experimental eggs were discriminable from natural robin eggs, yet these were never rejected. Cowbird eggs, which are rejected in 100% of experimental trials (Rothstein 1982, Briskie et al. 1993), were more similar in avian-perceived color than is generally understood in robin-cowbird literature, where cowbird eggs are often described as divergent from the eggs of their hosts. That the rejection rate of cowbird eggs was significantly higher than predicted based on the function derived from experiments with artificial egg colors demonstrates that egg rejection occurs specifically in response to cowbird eggs, despite their low discriminability from robin eggs, and suggests that parasitism by cowbirds may have influenced robins’ responses to the presence of parasitic eggs.

That I did not find a significant effect of achromatic contrast between robin and models eggs in predicting egg rejection may yet have been a result of a relative lack of achromatic variation among the model egg stimuli used in this experiment. Further investigation should include tests of achromatic contrast and achromatic cone catch in predicting responses to parasitism, based on an experimental protocol specifically designed to test the hypothesis that egg rejection can be predicted by differences in brightness between model and host robin eggs. For this experiment, it would be necessary to use model eggs which varied in achromatic, but not chromatic, reflectance. For example, artificial parasitism with model eggs ranging in brightness from white to black, including several eggs colored intermediate shades of gray, would allow for the brightness variation necessary to test for any effect on likelihood of egg rejection.

The use of avian visual modeling in brood parasitism research, together with full-spectrum reflectance measurements, has gained momentum in recent years, perhaps
owing to the significance of using biologically realistic sensory models to predict behavior and provide otherwise inaccessible insight into mechanisms, combined with the relative accessibility of these approaches. Measurement and analysis of spectral sensitivity data in non-human systems is a relatively new field, and important gains remain to be made in this, and in the application of these data to our interpretation of animal behavior. In particular, testing the relative importance of signal qualities not visible to humans (e.g. UV-reflectance and finely-tuned chromatic discrimination) in carrying signal information, across diverse taxa, can provide insight which was largely unavailable before the advent of such technology. Specific to brood parasites and their hosts, future study should be directed toward the reconciliation of differences between known spectral sensitivities and observed behavior.

I look forward to the widespread use of microspectrophotometry in describing spectral sensitivities of a broader range of taxa, and individuals within the same species. For example, evolved inter-individual or inter-sexual differences in spectral sensitivities (Fernández-Juricic et al. 2013), for hosts of mimetic brood parasites, may drive differential responses to brood parasitism within species, such that some individuals are better able to discriminate parasitic eggs, and all eggs that are discriminable are in fact rejected. In this case, egg rejection could be constrained by existing sensory biases within a given population, lending advantage to individuals able to better discriminate foreign eggs due to retina physiology, and representing active selection on the composition of the retina itself. Additionally, differences in photoreceptor sensitivities in males versus females may exist as a product of differential nest attendance and incubation, such that in species where only the female incubates, she is better able to discriminate foreign eggs.
than is the male. In species where incubation is shared, we would expect no difference in sensitivities, if selective pressure from brood parasitism does indeed drive the spectral sensitivities of host birds. Notably, for cowbirds, females have lower chromatic and achromatic resolution than do males (Fernández-Juricic et al. 2013) – comparing male versus female cowbird spectral sensitivities to those of their hosts may provide interesting tests of the value of visual discrimination abilities to the brood parasitic strategy.

With the widespread use of microspectrophotometry, we can not only model the effects of specific signal attributes on behavior of a receiver in a specific niche, but we can gain a greater understanding of the magnitude of variation in such attributes within populations, and investigate how this contributes to the decisions and relative successes of each (Fernández-Juricic et al. 2011). In my research, I did not address whether individual differences in behavior exist due to differences in sensory physiology, and it remains possible that the degree of tolerance of unlike eggs demonstrated in our study population is a result of individual differences not only in decision-making, but in photoreceptor sensitivities themselves between acceptor and rejecter individuals. The demonstrated individual repeatability of egg rejection behaviors in parasite-hosts (Ch. 4, also Samaš et al. 2011) are consistent with such a scenario.

In another line of future studies, microspectrophotometric measures could be used to address potential differences in spectral sensitivities between hosts of mimetic versus non-mimetic brood parasites, by measuring the specific chromatic sensitivities of a wide range of brood parasite hosts, of diverse lineages. In addition to the aforementioned comparisons, these data would allow for comparative phylogenetic research investigating the effects of phylogenetic bias in determining host responses to parasitism. For example,
many Turdus species reject experimental parasitic eggs, even though they are unsuitable brood parasite hosts (Grim et al. 2011). Further study should include a character state reconstruction of the species-rich Turdus clade, in order to test the alternative hypothesis that egg rejecter status in American robins, European blackbirds, and song thrush represents a behavior inherited from an egg-rejecting common ancestor. These would enable a comprehensive multispecies comparative study of sensory coevolution (sensu Anderson et al. 2009).

Likewise, further consideration should be given to the collection and handling of spectrophotometric data. Subtleties in measurement such as probe angle and distance from the measured substrate can significantly impact results, particularly for measures of brightness (RC pers. obs.). Additionally, spectral measures generate large amounts of data, which present a challenge to even the most organized researchers. Researchers interested in animal coloration would benefit from a standardized framework for collecting and managing this data, and organizing it for analysis. In addition, the construction of an online data bank, similar to GenBank, would both facilitate the sharing of data, and enable increased transparency in methodology. Perhaps the requirements that all raw data used for publications in some peer-reviewed journals and government funded research will alleviate this gap sooner.

In Chapter 3, I experimentally examined costs associated with rearing a parasitic cowbird chick alongside a robin brood, seeking to confirm a critical assumption underlying the evolution of cowbird-specific responses to parasitism. If robins’ ability to recognize and reject parasitic eggs has evolved in response to parasitism by cowbirds,
there must have existed some cost to robin hosts that is recoverable through the removal of these eggs (‘recoverable cost’, *sensu* Servedio and Hauber 2006). By cross-fostering cowbird chicks in robin nests according to several treatments, designed to disentangle species-specific effects from effects that exist as a byproduct of the timing of cowbird parasitism, I have found that cowbird parasitism is costly for both robins and for the cowbirds themselves. Robin fledging success decreases as a result of parasitism, yet cowbirds reared in competition with robin chicks are themselves significantly less likely to survive than are control cross-fostered robin chicks. The specific results suggest a cost of parasitism at the nestling stage, which may explain the evolution of defenses specifically against cowbird eggs observed in our earliest study. More difficult to explain, however, is that robin defenses against parasitism seem to be in place while the cowbirds have not evolved egg mimicry to combat host response. There is also no conclusive information, pro or con, whether cowbirds avoid parasitizing robins (Strausberger and Ashley 1997), despite that corearing is more detrimental to cowbirds than to robin chicks. This may be an indirect result of the cowbirds’ generalist strategy, as this would dilute the selective pressure on cowbirds to avoid failed parasitism on robins. Robins, though less aversely affected by parasitism than the cowbirds themselves, may have experienced stronger selective pressure due to this dilution.

In solving the remaining pieces of this puzzle, it is important to learn whether cowbird parasitism also imposes recoverable costs on robin hosts in the fledgling stage. Recent work has shown that often host parents are seen feeding only cowbird chicks (Rasmussen and Sealy 2006), and post-fledging costs are known to affect baywings (*Agelaioides badius*), hosts of screaming cowbirds (*Molothrus rufoaxillaris*; De Marsico
et al. 2012). Likewise, cowbird parasitism may impact hosts’ residual reproductive success, as compared to rearing the equivalently-sized host brood (Hauber 2002, 2006). GPS or radio tracking of fledglings, though challenging, would enable researchers to determine whether costs of parasitism are also imposed outside of the nest, and, critically, impact residual reproductive value (Hauber 2002, 2006). Such investigations can provide tests for the prediction that, for example, egg rejection is more common among cowbird hosts experiencing greater post-fledging costs of parasitism. This provides a critical test for determining why and how egg rejection exists in the subset of hosts appearing to have the least need for such defenses.

In Chapter 4, I have addressed a second important assumption underlying brood parasite-host coevolutionary theory, namely that of the evolvability of egg rejection responses to parasitism. As a first approach, I investigated the repeatability of rejection in response to sequential parasitism. Measures of repeatability are relevant to the evolution of responses to parasitism because only phenotypes that are consistent within individuals, but have some variation between individuals (and are thus, by definition, repeatable), can respond to selection (Bell et al. 2009). By definition, this measure represents the maximum influence that either genetic or environmental factors can have (when the other is 0; Boake 1989) on a given outcome (but see Dohm 2002). My experiments here revealed that repeatability of response to parasitism in egg rejection within the same nesting attempt is high, with most individuals consistently rejecting sequentially presented parasitic eggs. Though repeatable responses to parasitism may also occur as a learned response to prior parasitism, and therefore repeatability estimates cannot directly
inform estimates of heritability _per se_, that responses to simulated parasitism are repeatable may provide evidence of the feasibility of genetic evolution of egg rejection in response to selective pressure from cowbird parasitism in our population.

The importance of repeatability is often not explicitly addressed, particularly in brood parasite research, although recent efforts have increasingly focused on this trait (Vikan et al. 2009, Samaš et al. 2011; also see Shaw and Hauber 2009, 2012). For hosts of brown-headed cowbirds, with strong bimodality in their responses to parasitism, investigating repeatability can yield low measures, despite the high rate of recurrence of a given behavior, as a result of very low population-level variation. A crucial component of my repeatability study was in the use of model eggs which were rejected at intermediate ( _sensu_ Røskaft et al. 2002) rejection rates. This not only allowed for meaningful measure of repeatability, but also served as a useful reminder that bimodality in response to a single stimulus does not preclude differential responses to other, similar stimuli, which may be generalizable in ways allowing for further insight toward evolutionary mechanisms. This, in and of itself, warrants further experimental investigation, as it may help to illuminate the steps in the evolutionary pathway leading to obligate brood parasitism and egg rejecting hosts.

A shared coevolutionary history is predicted to influence the color of an egg-rejecting host’s eggs, particularly when parasitic eggs mimic host eggs, in order to reduce the likelihood of committing errors in egg recognition. Hosts of egg-mimicking parasites can evolve reduced intraclutch color variation in order to facilitate correct recognition, and increased interclutch color variation occurs within populations as a byproduct of this
constraint. With greater difference in appearance between host and parasitic eggs, the relative importance of inter- and intraclutch egg color variation in determining responses to parasitism should decrease (Stokke et al. 2007), so for hosts of non-mimetic brood parasites, selective pressure to avoid misidentification is not an important factor influencing the evolutionary trajectory of egg color variation. In the final Chapter 5, I address the predicted adaptive changes in host egg color variation in response to parasitism by either a non-mimetic, or conspecific (functionally mimetic) brood parasite. This question was previously unaddressed for hosts of non-mimetic brood parasites, except for a single experiment (Peer et al. 2010) and now, a comparative analysis (Abernathy and Peer, in press), due to the lack of variability in cowbird host responses to parasitism by cowbird eggs (all eggs rejected). Here, by parasitizing with an intermediately-rejected blue model egg, I was able to collect new data with meaningful behavioral variation in egg rejection responses, to address predictions of this theory explicitly.

I predicted that if robin egg rejection is the result of historical parasitism by conspecifics, egg color should vary significantly less within a single clutch than between clutches. Similarly, in response to experimental increase in egg color variation, rejection rates for parasitic eggs should decrease. If robin egg rejection is the result of historical parasitism by cowbirds, however, intraclutch egg color variation should not significantly differ within versus among clutches, and the ability to reject parasitic eggs should not change in response to manipulation of color variation within clutches. I found that the degree of clutch color variation was greater between clutches than is predicted by variation within clutches, yet, experimentally manipulated clutch color variation did not affect rejection rates. These results support egg rejection as an evolutionary response to
both mimetic and non-mimetic brood parasites. I discuss these findings in the context of
cognitive mechanisms underlying egg rejection, and conclude that for hosts of non-
mimetic parasites, and the potential role of cognitive mechanisms in mediating
differential responses to parasitism among these hosts.

In its entirety, this project has provided support for the scenario wherein cowbird-
robin interactions directly influence robins’ responses to parasitism by cowbirds – that is,
robin egg rejection is most readily explained by their exposure to parasitic cowbirds. My
research has also opened up several avenues for continued investigation, which may
serve as important components of a full understanding of the effects of sensory
mechanisms on the evolution of brood parasite host behavior.

This body of research has highlighted the need for increased attention to the
specific triggers and mechanisms of host responses to non-mimetic cowbird parasitism,
particularly as there is much yet to be revealed through further examination of the
sensory drivers of egg rejection. In particular, whether variation in host responses to
parasitism occurs because of cognitive, sensory, or other inter-individual differences, is
among the most compelling questions remaining open at the end of my research program.
Further research should include revisiting the classic studies on egg rejection in robins,
for example, those of Friedmann (1929), Rothstein (1982), and Briskie et al. (1993),
using behavioral experimentation in combination with up-to-date sensory system
analyses (e.g. microspectrophotometry and avian visual modeling), in order to test
predictions stemming from sensory versus cognitive effects on individual differences in
response to parasitism.
In closing this chapter, I would like to extend my gratitude once more to my advisory committee, collaborators, and cohorts, the landowners around Ithaca, my various funding agencies, and of course, to the robins for their fascinating behavior.
References


