BEHAVIORAL CHARACTERISTICS AND HORMONAL MECHANISMS OF EGG EJECTION IN THE AMERICAN ROBIN (*TURDUS MIGRATORIUS)*

BY

ABBIGAIL MAY TURNER

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Biology
with a concentration in Ecology, Ethology, and Evolution
in the Graduate College of the
University of Illinois Urbana-Champaign, 2023

Urbana, Illinois

Doctoral Committee:

Associate Professor Phil Anderson, Chair
Professor Mark Hauber
Professor Michael P. Ward
Assistant Professor Eva Fischer
Dr. Jeff Hoover
Assistant Professor Mikus Abolins-Abols, University of Louisville

ABSTRACT

Avian obligate brood parasites lay their eggs in other species nests, effectively releasing themselves from many of the behaviors related to raising their own offspring. This, in turn, imposes moderate to severe costs on hosts to raise the unrelated progeny. Some hosts of obligate brood parasites accept the parasitic egg(s); however, several host lineages have evolved defense behaviors to curb the costs associated with brood parasitism. One of these defense behaviors is the ejection (i.e., removal) of foreign eggs from the host's nest. My dissertation focuses on the proximate cues, the behavioral characteristics, and endocrine mechanisms of anti-parasitic egg rejection in the American robin (*Turdus migratorius),* a well-known ejecter of parasitic brown-headed cowbird (*Molothrus ater)* eggs.

First, I reviewed the previously published literature on the sensory cues that robins use (or do not) to eject foreign eggs from their nests. Visual cues such as background eggshell color and maculation are all important sensory cues utilized by robins to inform rejection decisions. Through my review, I identified under-studied sensory modalities and cues (i.e., tactile and odor cues) and then set out to experimentally test these. Through these studies I found a significant effect of tactile but not odor cues on robin egg rejection where rougher egg surfaces had higher rejection rates.

The previous literature on robin egg rejection has largely focused on the egg rejection impact of the characteristics of the model egg(s) being added to experimental robin clutches. My remaining chapters, instead, aimed to understand the mechanisms and traits of this behavior more fully. We know little about how quickly and where (i.e., distance and direction taken) foreign eggs are deposited after the female has decided to remove it from the nest. Using a novel methodology of inserting radio-transmitters inside 3D printed model eggs, I examined the characteristics (i.e., latency, distance, and direction) of different colored model eggs ejected by the same female robins. I found egg color, but not female identity, to be a significant predictor of ejection latency while neither egg color nor female identity predicted the distance and direction ejected eggs were taken. Through further experimentation, however, I determined that female robins were not repeatable in where they deposited the egg but showed some consistency in how quickly they removed the egg and returning to the nest post-flushing when given the same model egg color repeatedly.

Finally, I experimentally tested for the role of corticosterone, a glucocorticoid hormone often released in response to environmental stressors, in robin's egg rejection behavior. Using a recently developed non-invasive method of corticosterone delivery, I found that experimentally increasing this glucocorticoid increased rejection rates of a model egg color that is typically rejected at intermediate rates. Taken together, the findings from these studies have significantly contributed and expanded our understanding of a well-studied anti-parasitic behavior in an avian host-brood parasite system.

ACKNOWLEDGEMENTS

I never imagined that I would go to graduate school. I will always be thankful to Dr. Dustin Reichard and Dr. Beth Schultz for showing me that I am fully capable of pursuing a graduate degree and that birds are simply the coolest study organisms. I'd like to thank my PhD advisor, Dr. Mark Hauber, for all of his support and encouragement throughout this life-changing journey. You've provided me with the perfect place to grow and learn as a biologist. Thank you for always challenging me and believing in my capabilities. I'd also like to thank my entire committee Dr. Michael Ward, Dr. Eva Fischer, Dr. Jeff Hoover, Dr. Phil Anderson, and Dr. Mikus Abolins-Abols for helping me to fine tune my ideas in a constructive way and providing so much knowledge and support to my dissertation. I'd like to thank my cowbird lab mates for their support throughout the years and for the immediate community I found in you. I have learned something valuable from each of you!

To my mom, Jamie, and my siblings, Clara and Elijah, I absolutely could not have done this without you. I hope you are as proud of me as I am of all of you. To my grandparents, Tom and Jean, who are probably my biggest cheerleaders on this earth, I am forever grateful for how hard you root for me. To my many remaining family and friends, I will never be able to adequately express how much your undying love and support has helped get me through this experience. I feel extremely lucky to have the community that I do and I am so grateful. Lastly, I'd like to thank my pet children Obi and Echo for bringing me so much joy and comfort that was essential for me throughout graduate school. Much love to you all!

To my late father who loved to learn and instilled that love in me. I wouldn't have made it here without you, dad.

"I know I can be what I want to be. If I work hard at it, I'll be where I want to be."

Peace

TABLE OF CONTENTS

CHAPTER 1: THE AMERICAN ROBIN (*TURDUS MIGRATORIUS):* A FOCAL SPECIES FOR ANTIPARASITIC EGG REJECTION STUDIES AMONG HOSTS OF THE BROWN-HEADED COWBIRD (*MOLOTHRUS ATER*)

ABSTRACT

Obligate avian brood parasites lay their eggs in the nest of other species and impose reproductive costs upon their hosts. In response, many hosts have evolved a variety of defenses to avoid or reduce the costs associated with parasitism, including the rejection of parasitic eggs. Here, we review the different sensory and contextual cues involved in egg rejection by the American robin (*Turdus migratorius*), one of the handful of rejecter host species of the brood parasitic brown-headed cowbird (*Molothrus ater*) in North America. Robins are an open cup nesting species whose eggs dramatically differ in appearance from the cowbirds' eggs; therefore, we expect that visual cues would be the primary sensory modality used when rejecting foreign eggs. The results of our comprehensive review confirm that visual cues, including eggshell color and maculation, are strong predictors of robins' egg rejection responses. Egg size, egg shape, and other contextual traits (including clutch composition, maternal status, and sympatry with the parasite) also affect egg rejection in this host species. Future research into the mechanisms would benefit from comparisons to other strongly and moderately egg-rejecter species of brown-headed cowbirds, as well as further seek to understand why so many hosts of this parasitic lineage accept foreign eggs. We also discuss the implications of this review for other egg rejecter and non-rejecter hosts of diverse avian brood parasites.

INTRODUCTION

Avian obligate brood parasites lay their eggs in the nests of other species (Friedmann 1929). Hosts of brood parasites often endure reproductive costs from raising unrelated offspring and in turn have evolved a variety of defenses to avoid fitness costs associated with parasitism (Rothstein 1975a). Indirect strategies to avoid brood parasitism involve breeding outside of the parasite's own breeding season (e.g., Anderson et al. 2013) or in cavities with entrance holes too small for the parasite to enter (Pribil & Picman 1997). In contrast, direct resistance strategies include frontline defense (*sensu* Feeney et al. 2012), whereby host species recognize and actively protect their nests from adult brood parasites before parasitism occurs (Briskie et al. 1992, Welbergen & Davies 2009). Other hosts abandon their nests altogether after being parasitized (Hosoi & Rothstein 2000) or, like yellow warblers (*Setophaga petechia)*, bury the entire clutch before rebuilding atop of it (Guigueno & Sealy 2009). Clutch uniformity, or limited color variation in clutch appearance, could aid in foreign egg recognition (Croston & Hauber 2015b) and, subsequently, the rejection of foreign egg(s) in the nest (Antonov et al. 2007). As one of the last lines of defense, some host species, such as the large-billed gerygone (*Gerygone magnirostris)*, can distinguish their own nestlings from their brood parasite, the little bronze cuckoo (*Chrysococcyx minutillus*), and eject the latter's young from the parasitized brood (Noh et al. 2018).

Egg rejection is the focal anti-parasitic defense strategy examined in this review but is a surprisingly rare host response to avoid parasitism costs imposed by the North American brown-headed cowbird (*Molothrus ater,* hereafter: cowbird; Winfree 1999). Successful egg rejection requires the accurate discrimination and recognition of the foreign egg in the clutch, and host species might tap into different cognitive mechanisms to accomplish it (Manna et al. 2017). True (or direct) egg recognition involves the use of a mental template whereby the hosts remember the appearance of their own eggs regardless of its majority in the clutch (Rothstein 1975b, Ban et al. 2013). An alternative recognition process suggests that hosts reject the egg that is most dissimilar from the majority of eggs (via discordancy) and do not necessarily recognize their own eggs *per se* (Rothstein 1975b, Moskát et al. 2014b).

Hosts of brood parasites can utilize diverse sensory cues to aid in foreign egg recognition. For instance, egg color varies greatly amongst the hosts of brown-headed cowbirds (Rutledge et al. 2021) and, therefore, can serve as an important visual cue for the parasitic egg that is typically distinct (non-mimetic) in color and maculation (or spotting pattern) from the hosts' own eggs (Rothstein 1982, Luro et al. 2018). Similarly, in reed warblers (*Acrocephalus scirpaceus),* a common host of a strongly-mimetic host-specialist race of the common cuckoo (*Cuculus canorus*), when model eggs that were painted more dissimilar in egg color from the hosts' own egg colors, they were rejected at higher rates (Davies & Brooke 1989). This effect has also been found in great reed warblers (*Acrocephalus arundinaceus)* (Abolins-Abols et al. 2019). Maculation is also an important visual cue for some host species, especially those with immaculate eggs, and is also often a strong predictor of egg rejection (Hauber et al. 2014, Dinets et al. 2015, Carmody et al. 2016, Dainson et al. 2017). For instance, in chalk-browed mockingbirds (*Mimus saturninus)*, maculation presence was a predictor of greater egg rejection rates of model shiny cowbird (*Molothrus bonariensis*) eggs, even when the eggshell's ground color was experimentally controlled for (Hanley et al. 2019). Egg shape can, too, serve as a visual and/or tactile cue for egg recognition; accordingly, yellow warblers reject star shaped model objects from their nest at higher rates than egg-shaped models (Guigueno & Sealy 2009). Clutch composition, specifically a more variable clutch appearance, favors the cowbird egg's acceptance in common grackles (*Quiscalus* *quiscula)* (Peer et al. 2010). Egg mass can be perceived as a tactile cue and is known to significantly modulate egg rejection in the Eurasian blackbird (*Turdus merula*), with heavier eggs accepted more than lighter and control-weighted eggs (Ruiz-Raya et al. 2015). Broken eggshells in the nest, presenting a sharp edge, can be a visual and/or tactile cue as well for nesting birds; accordingly, female red-winged blackbirds (*Agelaius phoeniceus*) rejected most simulated broken model eggs from their nests (Kemal & Rothstein 1988). Furthermore, olfactory cues may be used by Eurasian magpies (*Pica pica*) when rejecting foreign eggs (Soler et al. 2014) and could represent another sensory modality utilized by rejecter host species. Finally, there is now a suggestion for social mediation of egg rejection in at least one rejecter species of non-mimetic parasite eggs (Yang & Feeney 2020), which would parallel known patterns of socially-mediated antiparasitic mobbing among diverse host-parasite systems (Thorogood & Davies 2012, Feeney & Langmore 2013, Campobello & Sealy 2018).

American robins (*Turdus migratorius*, hereafter: robins) reject almost all cowbird eggs naturally or experimentally deployed in their nests (Friedmann 1929, Rothstein 1982) (but see Figure 1.1). Therefore, robins do not endure severe costs of brood parasitism, except for the cowbird's removal of a robin egg prior to parasitizing the nest (Croston & Hauber 2015a). Even when cowbird nestlings were cross-fostered into robin nests, the robin's egg-to-fledgling survival was not significantly impacted, whereas only ~50% of cowbird hatchlings survived to fledging (Croston & Hauber 2015a). This low brood parasite survival rate is likely due to the inability of cowbird nestlings to efficiently compete for parental care with their larger, more rapidly developing host nestmates (Croston & Hauber 2015a). Overall, robins almost never rear cowbird nestlings because female robins recognize and reject the cowbird egg by utilizing a suite of sensory cues to inform egg rejection decisions.

As the responses of robins to experimental brood parasitism has been studied so extensively, they provide an ideal focal species to productively explore the stimuli used to detect and reject parasitic eggs, and the limits to the perception of these stimuli within valid ecological and evolutionary contexts in the wild (Luro & Hauber 2020). Here, we review whether and how American robins rely more on certain sensory modes of egg recognition than others (Figure 1.2). Specifically, as an open cup nesting species whose eggs dramatically differ in avian-perceivable appearance compared to the parasite's eggs (see Figure 1.1), we are especially interested to see if robins rely on visual cues more than any other sensory modality to inform their own egg rejection decisions. Our review also provides a framework for studying recognition cues in other egg-rejecter hosts of avian brood parasites.

METHODS

For this overview, we sought out published papers from Google Scholar and Web of Science on robin egg rejection behaviors by conducting a search using the terms: "American robin" and "egg rejection" or "egg recognition" and selected those papers that assessed the use of a sensory trait/cue and its effect on the robin's egg rejection responses. Potential egg recognition cues were classified as: *egg-specific sensory traits, clutch composition, maternal traits, and spatial overlap.* We compiled information on egg recognition cues from published works into a table (Table 1.1) that included author(s), focal trait analyzed, whether the trait was experimentally manipulated, if the trait analyzed affected egg rejection for each of the experimental treatments/references identified. These were supplemented by sample sizes per treatment and odds ratios for treatment vs. control egg rejection patterns. Odds' ratios (OR) were provided as a standard metric of experimental effect sizes (experimental vs. control responses) to allow future comparisons of statistical and biological effects across studies. Specifically, although our study is not a formal meta-analysis, we also included OR for future such works. The OR values were calculated from the published studies using the following formula:

$$OR = AD / BC$$

Where A is the number of eggs rejected in the experimental treatment, B is the number of eggs rejected in the control, C is the number of eggs accepted in the experimental treatment, and D is the number of eggs accepted in the control.

RESULTS

Egg Traits

Ground Color

Egg color is by far the most often tested visual cue for egg recognition in American robins and is a strong predictor of egg rejection (e.g., Rothstein 1982, Croston & Hauber 2014a, Croston & Hauber 2015b, Igic et al. 2015, Dinets et al. 2015, Carmody et al. 2016, Hanley et al. 2017, Luro et al. 2018, Hauber et al. 2019). Robins lay immaculate blue-green eggs (see Figure 1.1) and are more likely to reject eggs that are either human-perceivably discriminable (Rothstein 1982, Carmody et al. 2016, Luro et al. 2018) or avian-perceivably deviate (Croston & Hauber 2014a, Igic et al. 2015, Hanley et al. 2017, Hauber et al. 2019, Hauber et al. 2020b) from this color. Specifically, eggs on the more white-beige/browner side of the natural avian eggshell-color spectrum (*sensu* Hanley et al. 2015) elicited higher rejection rates than perceivably similarly distinct but bluer eggs (Croston & Hauber 2014b, Hanley et al. 2017). These findings on the role of blue (accepted) vs. brown/beige coloration (rejected) have been also consistent in a study examining the blunt pole hypothesis (Polačiková & Grim 2010); however, in robins, Hauber et al.

(2021a) rejected directional predictions that robins gather more information on egg characteristics from the blunt-pole (vs. the sharp-pole) of the foreign egg's surface.

As expected, model eggs that are painted to mimic natural robin egg color are accepted at high rates (Rothstein 1982, Dinets et al. 2015, Igic et al. 2015, Carmody et al. 2016, Luro et al. 2018). Furthermore, manipulating the variation in coloration among eggs within a robin's own clutch does not affect rejection behavior of a focal model egg (Croston & Hauber 2015b; also see Clutch Composition section below). Similarly, there is no evidence that robins tap into their own, individually-consistent natural egg-coloration in a clutch and compare a model egg's avian perceivable color distance from the natural robin eggs' appearance for rejection decisions (Hauber et al. 2020b). Thus, there is no support for the self-referencing hypothesis in robin egg rejection mechanisms (*sensu* Stevens et al. 2013, Hauber et al. 2015). In addition, robins are able to perceive the avian eggshell's ultra-violet (UV) region of reflectance (Aidala et al. 2012); however, neither of two studies reports that UV-blocked egg colors affected rejection rates relative to controls (Croston & Hauber 2014a, Abernathy & Peer 2015).

Maculation

The maculation, or spotting pattern, of an egg can be an especially important visual cue for robins and other species whose eggs are naturally immaculate (e.g., Hauber et al. 2014, Dinets et al. 2015). The robin's natural parasite, the brown-headed cowbird, lays eggs that are noticeably different in appearance as they are creamy-white in shell ground color with prominent brown maculation (Figure 1.1). Thus, maculation has been established as a critical visual cue for robins when rejecting foreign eggs (Rothstein 1982, Dinets et al. 2015, Carmody et al. 2016, Dainson et al. 2017, Luro et al. 2018). The presence of maculation alone (vs. absence), can be a strong visual cue for egg rejection. For example, Luro et al. (2018) found that robins reject robin-sized maculated model eggs 68% more than robin-sized immaculate model eggs painted with the same robin-mimetic blue-green background color. The color of the spotting itself can also be informative; in the only study which addressed this variation in hosts of avian brood parasites, model eggs with increasingly greater degrees of chromatic contrasts in spotting vs. ground coloration (when that ground color was robin-mimetic blue) were increasingly more likely to be rejected (Dainson et al. 2017).

Shape

Small, subtle variations in egg shape could be perceived by visual and/or tactile modalities but are not predictors of egg rejection when the model egg closely resembles the overall size of natural cowbird eggs (Underwood & Sealy 2005, Igic et al. 2015). In turn, clearly non-egg like egg shapes are associated with high rejection rates (Underwood & Sealy 2005, Hauber et al. 2021b). Experiments conducted with cylindrical or cuboid shaped white model eggs increased rejection rates by 59.5% (Underwood & Sealy 2005). Similarly, the likelihood of egg rejection increased when panel length and width of model eggs were stretched creating a series of increasingly un-egg like shapes of robin-blue painted models (Hauber et al. 2021b). Deviations from natural egg shapes could be perceived as nest debris and elicit prompt sanitation behaviors like the removal of leaves, flowers, broken eggshells, or fecal sacs from the nest cup (Guigueno & Sealy 2012). The nest sanitation hypothesis predicts that rejectors of brood parasitic eggs should also have high rates of nest sanitation (Rothstein 1975a), however, Luro and Hauber (2017) found no support for this hypothesis in robins rejecting a non-mimetic deep-blue cowbird sized egg. They found rejection of this egg type to be intermediate, individually repeatable, but also independent of the same robin's nest sanitation pattern of a deep-blue painted foliage-fragment in the nest (Luro & Hauber 2017).

Size

Egg size can serve as a visual and/or tactile cue for egg recognition. Except for a handful of cases, where visual perception is excluded in the full darkness of the enclosed host nest (e.g., rufous horneros (*Furnarius rufus*): Tosi-German et al. 2020), it remains unclear whether hosts perceive this trait through vision and/or physical contact. Compared to other egg characteristics, robins rely less on size as a sensory cue for egg recognition. Multiple studies in our analysis did not find a statistically significant effect of size on egg rejection patterns (Friedman 1929, Rothstein 1982, Igic et al. 2015). Nonetheless, Rothstein (1982) detected that differences in model egg size significantly altered the latency to rejection through smaller eggs being rejected more swiftly. Similarly, cowbird-sized egg models were more likely to be rejected than robin-sized models (Carmody et al. 2016, Luro et al. 2018) which may indicate that robins are specialists on cowbird-sized eggs' rejection.

Egg-Nest Visual Contrast

Visual contrasts between eggs and the substrate upon which they are deposited (e.g., nest lining) (Endler & Mielke 2005) may be used for egg recognition. Contrary to this idea, Aidala et al. (2015) found that red, beige, and mimetic robin-colored model eggs were all rejected at similar relative rates to eggs, regardless of natural or experimental nest background colors, indicating a lack of support for this hypothesis and its implied egg-rejection mechanism in robins.

Spatial Chromatic Contrast

Egg maculation color and patterning, combined with egg background coloration are multicomponent visual cues that birds may use when viewing and recognizing foreign eggs in their nests (Hanley et al. 2019). Accordingly, in a recent experimental study where robins were presented with checkered model eggs with a range of blue and yellow square sizes, robins were more likely to reject model eggs with larger spatial chromatic contrasts (i.e. model eggs with larger squares) (Luro et al. 2020), indicating both spatial and chromatic contrast together may represent a visual cue for robins when making egg rejection decisions in the presence of maculation and its contrast against the eggshell's ground coloration (also see Dainson et al. 2017).

3D Model Egg Material

Many of the studies in our review utilized artificial model eggs when conducting rejection experiments. Previous studies on egg rejection used model eggs constructed from plastic, wood, plaster-of-Paris, and, most recently, 3D printed natural plastic eggs that offer a more precise design that can be easily manipulated (Igic et al. 2015, Hauber et al. 2021b). Despite these advantages of 3D printing, there was no difference in rejection rates based on the type of model egg used (plaster vs. 3D printed) (Igic et al. 2015). Furthermore, real cowbird eggs are rejected at the same rates as model cowbird-colored and maculated eggs (Croston & Hauber 2014a).

Clutch Composition

Clutch Size

On average, robins lay 3-4 eggs per clutch (Abolins-Abols & Hauber 2020a). The number of eggs present at the time of parasitism (natural or experimental) can serve as a visual and/or tactile cue for egg rejection. Yet, the two relevant studies in our review have conflicting results for the effect of clutch size on egg recognition cues. Hauber et al. (2019) found rejection rates were significantly lower in experimentally parasitized robin nests with three natural robin eggs in the nest compared to four, as the final clutch size. In contrast, robins with smaller final clutch sizes were more likely to reject the model egg (Abolins-Abols & Hauber 2020a). Both of these studies focused on the completed clutches during the early incubation period, but the non-mimetic model eggs used by Hauber et al. (2019) were beige in color, whereas those in Abolins-Abols and Hauber (2020a) were deep-blue in appearance. These conflicting results indicate a need to further and experimentally examine the role of how clutch size interacts with model egg rejection patterns in robins.

Direct Recognition vs. Discordancy

The direct ("true") egg-recognition hypothesis states that brood parasite hosts can recognize their own eggs within a clutch from foreign egg(s) regardless of which egg type is the majority (or the totality) in the nest (Rothstein 1975b). In support of this true recognition scenario tested in robins' egg rejection, the number of parasite (brown-headed cowbird) relative to host eggs did not affect egg rejection when tested in partially (Lang et al. 2014) or fully replaced clutches (Rothstein 1975b).

The alternative to direct egg-recognition is the discordancy hypothesis, which states that birds can recognize foreign eggs because they are different from those eggs in the majority of the clutch, and therefore they stand out (Rothstein 1975b). However, even when cowbird-like model eggs were the majority of the clutch, robins continued to reject these, thereby eliminating the relevance of the discordancy hypothesis for this species (Lang et al. 2014).

Olfaction

Few studies have examined how the scent of the parasitic eggs can inform rejection decisions by rejecter hosts (but see Rasmussen 2013, Soler et al. 2014). The only study that has tested the effect of scent in robins found no effect of scent treatments (artificial: citrus, natural: cowbird cloacal lavage, control: solvent only) on egg rejection responses (Hauber 2020).

Maternal Traits

In robins, only females are known to recognize and reject foreign eggs from the nest (Scharf et al. 2019). However, in other species, including northern orioles (*Icterus glabula),* vinous-throated parrotbills (*Paradoxornis webbianus),* and European blackbird males have been observed recognizing and rejecting foreign eggs from the nest (Sealy & Neudorf 1995, Lee et al. 2005, Ruiz-Raya et al. 2019).

Maternal Characteristics & Physiology

Female physiology, both before and after the onset of parasitism, may affect egg rejection decisions. Corticosterone is a glucocorticoid hormone secreted in response to internal or external social or environmental stressors (MacDougall-Shackleton et al. 2019) and has been putatively linked to host defenses against brood parasites (Abolins-Abols & Hauber 2018, Ruiz-Raya & Soler 2020). Accordingly, in a correlative study, Abolins-Abols and Hauber (2020a) found a negative relationship between circulating corticosterone prior to parasitism and the likelihood of the subsequent rejection of a non-mimetic deep-blue model egg. Seemingly in contrast, robins experimentally injected prior to the onset of experimental parasitism with mitotane, a glucocorticoid inhibitor, were found to be more likely to accept the same type of non-mimetic, deep-blue colored model egg than robins that received a vehicle injection (Abolins-Abols & Hauber 2020b). Thus, naturally higher circulating (baseline) corticosterone appears to inhibit egg rejection responses, whereas the inability to synthesize corticosterone interferes with egg rejection. To reconcile these results further, two decisive experimental studies, therefore, should address whether (1) robins mount a glucocorticoid response when viewing non-mimetic (vs. mimetic) model eggs in the nest, and (2) experimentally raised circulating glucocorticoid levels cause increased or reduced subsequent egg rejection rates.

Furthermore, other maternal characteristics may affect a female's ability to reject foreign eggs. Accordingly, body mass of adult robins is weakly negatively correlated with egg rejection, with lighter females more likely to reject model eggs (Abolins-Abols & Hauber 2020a). Female age and/or ecological experience with brood parasites may also influence egg rejection behavior (Abolins-Abols & Hauber 2020a). Older female hosts of other species reject foreign eggs at higher rates than younger females (e.g., Lotem et al. 1995, Moskát et al. 2014a, Molina-Morales et al. 2014), although host age did not influence egg rejection outcomes in robins (Abolins-Abols & Hauber 2020a). Nonetheless, the determination of age by plumage can be imprecise in older female robins (after second-year plumage) and may have impacted these authors' inability to find a pattern with female age and egg rejection (Abolins-Abols & Hauber 2020a).

Maternal Investment in the Egg(s)

In the context of egg rejection, the maternal investment hypothesis predicts that females which invest more in the clutch should be more likely to reject foreign eggs (Hauber et al. 2020a). Nonetheless, except for some yolk corticosteroid concentrations (i.e., higher deoxycorticosterone levels), greater maternal investments such as earlier laying date, larger clutch size, more yolk steroids, and heavier yolk mass were not associated with more frequent egg rejection of the non-mimetic deep-blue model egg type (Hauber et al. 2020a).

Methods of Egg Rejection

Rejector cowbird hosts eliminate foreign eggs mainly by puncturing the egg and/or physically removing the egg from the nest via grasp ejection (Rasmussen et al. 2009). Other, smaller cowbird egg rejectors may be limited by bill morphology (i.e., gape size) and, thus, take a different approach to egg rejection by burying the clutch or by abandoning the nest (Guigueno et al. 2014, Hosoi & Rothstein 2000). Video recordings indicate that grasp ejection is the primary method of rejection of cowbird eggs by robins although pierce (puncturing of the egg) ejection also occurs (Rasmussen et al. 2009).

Repeatability

American robins are among the few species of brown-headed cowbird hosts that consistently (>90% of the time) reject this parasite’s eggs (Rothstein 1982), making it impossible to calculate repeatability due to the lack of sufficient acceptances. However, artificial model egg colors of yellow, red, and dark blue, are known to elicit intermediate rates of egg rejection and, thus, can be used to study the repeatability of rejection decisions at the individual level (Croston & Hauber 2014b, Luro & Hauber 2017). Accordingly, among the nests parasitized with these model egg colors, 81% of females rejected the foreign egg at both times they were experimentally parasitized indicating that individual egg rejection decisions are strongly repeatable in robins (Croston & Hauber 2014b). Similarly, Luro and Hauber (2017) found 94% of robin females repeated their egg rejection response when presented with the non-mimetic, deep-blue colored model eggs.

Lateralization

Lateralization is the specialized use of the left or right hemisphere of the brain which commonly occurs in birds when processing social (conspecific or predatory) visual information (Franklin & Lima 2001). A study in robins found that visual lateralization was dependent on the color of the model foreign egg, whereby mimetic eggs generated a lateral viewing bias but non-mimetic eggs were examined by both eyes (Scharf et al. 2019).

Spatial Overlap

Comparing populations of robins that occur in sympatry or allopatry with cowbirds provides a chance to study how the absence of brood parasitism affects the prevalence and, perhaps, the evolution of antiparasitic egg rejection in this system. At higher latitudes or at higher elevations, where cowbirds were absent, robins still rejected the cowbird eggs above background levels (Briskie et al. 1992, Carmody et al. 2016), although rejection rates were lower than in sites where cowbirds were present (Briskie et al. 1992, Carmody et al. 2016). These findings indicate that robins can still recognize foreign eggs in the absence of their brood parasite (Carmody et al. 2016), but that there is likely also a developmental plasticity, experience-dependence and/or a gene-by-environment interaction component to egg rejection behaviors (Briskie et al. 1992). Critically, to date no one has tested whether social influences (e.g., the neighboring female's egg rejection propensity) play a role in individual robins' own egg rejection propensities (*sensu* Yang & Feeney 2020).

DISCUSSION

Our expectation that American robins rely most on visual cues when making egg rejection decisions is supported by the comprehensive results of our qualitative overview. Visual cues, specifically egg color and maculation, are not only the most often tested but also consistently strong predictors of robin foreign egg rejection responses compared to other, strictly non-visual traits, including olfaction (Table 1.1). Nevertheless, robins also use other sensory modalities as well as maternal and perhaps brood parasitic experience-dependent cues to inform egg rejection responses. Egg size and shape could serve both as visual and/or tactile cues, however, robins rely less on these cues compared to strictly visual egg coloration and maculation cues when making rejection decisions. Differences in egg size may be more informative in species where the nest is completely dark and visual inspection of the egg is not possible (Tosi-German et al. 2020). Further, subtle deviations from a natural egg shape did not affect egg rejection (Igic et al. 2015, Underwood & Sealy 2005). In turn, several maternal traits can affect the likelihood of a female rejecting foreign eggs. Female physiology, specifically the level of circulating corticosterone levels, can influence a female's egg rejection decision (Abolins-Abols & Hauber 2018, 2020a, 2020b).

Little is known about the general role of olfactory cues in antiparasitic egg recognition (but see Soler et al. 2014). From our review of robins' responses to (model) cowbird eggs, olfaction did not influence egg rejection in robins; in the only study analyzing olfactory cues that we sourced for this review, neither unnatural scents of model eggs (citrus, human) nor a natural scent (cowbird cloacal lavage) affected egg rejection in robins (Hauber 2020). This was contrary to findings in magpies, where Soler et al. (2014) found that the unnatural scent treatment of human handling or tobacco smoke both significantly increased egg rejection rates, although natural scent treatments of uropygial secretion of cuckoos and cloacal scents of cuckoos and magpies did not do so.

Robins almost always reject natural whitish and spotted brown-headed cowbird eggs (Rothstein 1982, Rasmussen et al. 2009). Robins also overwhelmingly reject model eggs with cowbird-like white/beige ground colors (Croston & Hauber 2014a, Luro et al. 2018, Rothstein 1982) and eggs with heavy, brown maculation (Carmody et al. 2016, Luro et al. 2018, Rothstein 1982, Dainson et al. 2017). Even though robins seem to rely less on subtle shifts in egg size as a sensory cue for egg rejection (Igic et al. 2015), cowbird-sized eggs nonetheless elicit high rejection rates (Carmody et al. 2016, Luro et al. 2018). In addition, robins accept nearly all mimetic eggs in every experimental nest parasitism study, irrespective of size (Carmody et al. 2016, Dinets et al. 2015, Igic et al. 2015, Luro et al. 2018, Rothstein 1982, Hauber et al. 2019). These results suggest that robins specialize in cowbird egg rejection, as well as accepting eggs that mimic their own egg colors. To anticipate the rejection responses of robins against an invasion of a geographically novel brood parasite, Dinets et al. (2015) parasitized American robins in California with one of two model egg types that mimicked the size and coloration of two host-races of a European parasite, the common cuckoo. They found that robins rejected model cuckoo model eggs when these were maculated (as are of those cuckoos that egg mimic meadow pipits (*Anthus pratensis*)) and accepted model eggs when these were immaculate blue (as are those of cuckoos that mimic common redstarts (*Phoenicurus phoenicurus*)), which further implies that robins are specialists at rejecting maculated, more cowbird-like eggs (Dinets et al. 2015).

Many organisms use multicomponent and/or multimodal signaling (Hebets et al. 2016). The cues robins use during egg recognition are both multimodal and utilize many components within each of those sensory modalities. In robins the visual modes of recognition are most relied upon, but may also include tactile and, probably, not olfactory modalities. For example, egg size and shape are both components that can be accessed by robins within the visual and/or tactile modality of recognition, whereas egg color and maculation are strictly visual cues. In turn, different sensory cue components can serve as different salient cues in each respective modality (Table 1.1).

Implications for Cues and Mechanisms of Egg Recognition/Rejection in Other Host Species

Hosts whose brood parasite lays strongly mimetic eggs might utilize different cues when recognizing foreign eggs or nestlings. For example, high levels of mimicry by specialist parasites have been demonstrated in the *Vidua* parasitic finches where the parasite closely mimics their host nestlings visually, vocally, and posturally (Jamie et al. 2020). Due to such specialized mimicry, the hosts of these brood parasites may be either (1) constrained by this when recognizing foreign eggs or (2) use other sensory cues, such as olfactory or contextual traits rather than visual perception, to recognize their nestlings from the those of the parasite.

In turn, hosts with completely dark nests, like those of rufous horneros, cannot rely on visual cues of egg traits for egg recognition/rejection and have been shown to reject shiny cowbird eggs by size (Tosi-Germán et al. 2020). Other cavity nesting species, even if their nest is not completely shaded from light, may also use cues other than generic light reflectance when recognizing/rejecting foreign eggs (but see Manna et al. 2020). Accordingly, Avilés et al. (2006) has shown that cavity nesting species have higher ultraviolet (UV) reflectance in their eggs than semi-cavity and open nesting species, suggesting a role of egg UV reflectance in egg recognition. As predicted, spotless starlings (*Sturnus unicolor)* retrieved UV reflecting eggs from outside the nest, and they did not reject UV blocked model eggs from the nest (Avilés et al. 2016). Little is known about what other cues can inform egg recognition and/or rejection in the nest of cavity nesting species and further studies are needed to assess the alternatives in darker nest environments (e.g., Medina & Langmore 2019).

Future Directions in Studying Egg Rejection Behaviors

To our knowledge, no studies have designed a specific way to discriminate between the use of tactile vs. visual cues during egg rejection in an open cup nesting species. Such a distinction between the use of tactile vs. visual cues will be important to generate in future studies to accurately determine the relative set of sensory modalities upon which egg rejecter species rely.

Robins are among the few brown-headed cowbird host species that strongly reject foreign eggs (Friedmann 1929, Rothstein 1975a, Rohwer & Spaw 1988, Winfree 1999). Nevertheless, there are several other cowbird egg-rejecter host species with different mechanisms of rejection (i.e., egg burial, egg ejection, nest abandonment; Guigueno & Sealy 2009, Haas & Haas 1998, Peer et al. 2000, Underwood & Sealy 2005). For instance, yellow warblers have beige colored and brown-maculated eggs similar to those of the cowbirds (Rutledge et al. 2021) and may reject cowbird eggs by burying the parasitized nest and building a new nest atop of the old one (Guigueno and Sealy 2009). In turn, western meadowlarks *(Sturnella neglecta)* again lay beige eggs with some brown spotting and have been shown to reject 78% of artificial and natural cowbird eggs when experimentally parasitized (Peer et al. 2000). Gray catbirds (*Dumetella carolinensis)* lay dark blue-green eggs and show similar rejection rates of odd-shaped or non-mimetically colored model eggs as robins (Underwood &Sealy 2005). Lastly, brown thrashers (*Toxostoma rufum)* are another known rejecter species and eject cowbird eggs about 58% of the time when experimentally parasitized (Haas & Haas 1998). To our knowledge, little work has been done on the relative roles of diverse sensory modalities used by these and other intermediate-rate cowbird-egg rejecter species.

Most hosts of cowbirds accept parasitic eggs (Rothstein 1975a,b, Winfree 1999) and pay the associated fitness costs (Hauber 2003b). Thus, the question remains of why so many host species accept parasitic eggs when they differ significantly from their own? The evolutionary lag hypothesis predicts rejection behaviors have not had enough time to evolve in currently accepting host species even though raising parasitic young is costly (Ward et al. 1996, Winfree 1999). In contrast, the cost-benefit equilibrium hypothesis predicts hosts accept parasitic eggs because rejection is maladaptive (Soler et al. 1995, Winfree 1999). In other words, for some species, it could be less costly to raise the parasitic nestling rather than reject it (Soler et al. 1995). Still, there are limited studies on the sensory and cognitive mechanisms behind cowbird-egg acceptance behaviors per se. For example, when applied to the context of egg rejection, the optimal acceptance threshold theory may explain the variation in egg rejection decisions among hosts of brood parasites (Reeve 1989, Scharf et al. 2020). This acceptance threshold can be flexible depending on prior parasitism experience and has been demonstrated to experimentally be shifted in great reed warblers (Hauber et al. 2006). Furthermore, acceptors may still possess the ability to recognize foreign eggs in their clutches, especially hosts with eggs that differ significantly in appearance compared to their parasite’s eggs, yet do not to remove them due to other factors (e.g., ultimately, due to low parasitism rates or retaliatory behavior from the parasite: Ruiz-Raya and Soler 2020, or proximately, due to a lack of glucocorticoid responses mounted when viewing foreign eggs in the nest: Scharf et al. 2021a). Future research should also seek to narrow the gap in our understanding of the widely documented acceptance behaviors amongst the hosts of cowbirds, and other avian brood parasites in general.

CONCLUSION

By examining different cues for egg rejection in the same host species, the American robin, we can better understand the complexity of how different sensory modalities are utilized when making foreign egg decisions. Other cowbird-egg rejecter host species may have different egg characteristics, rejection methods, and life histories that necessitate the use of different cues when rejecting foreign eggs (cowbird and model eggs (Peer & Sealy 2004a)). For instance, olfactory cues did not significantly affect egg rejection in robins (Hauber 2020), whereas using foreign scent or unusual tactile qualities of nest contents could be more important cue for rejecter species whose eggs are closely mimicked by the parasite, or species with domed or cavity nests whose lighting milieu makes visual inspection of the eggs more difficult compared to open-cup nesting hosts. Therefore, the extensive literature we have gathered on egg, maternal, and habitat traits influencing egg rejection in robins should be replicated in other rejecter species of cowbirds and, in general, of other brood parasitic species (Hauber et al. 2015).

ACKNOWLEDGEMENTS

For funding, we thank the Harley Jones Van Cleave Professorship (to MEH) and a Graduate College Master's Student Fellowship (to AMT) at the University of Illinois at Urbana-Champaign. During the preparation of the manuscript, MEH was supported in part by the Hanse-Wisseschaftskolleg (Institute for Advanced Study), Germany. For discussions, we thank M. Abolins-Abols, N. Antonson, J. Hoover, A. Luro, H. Pollock, S. Winnicki, and many other colleagues.

FIGURES

Figure 1.1. A naturally parasitized American robin nest (robin egg: left and bottom) by the brown-headed cowbird (right). Photo credit: M. Hauber.

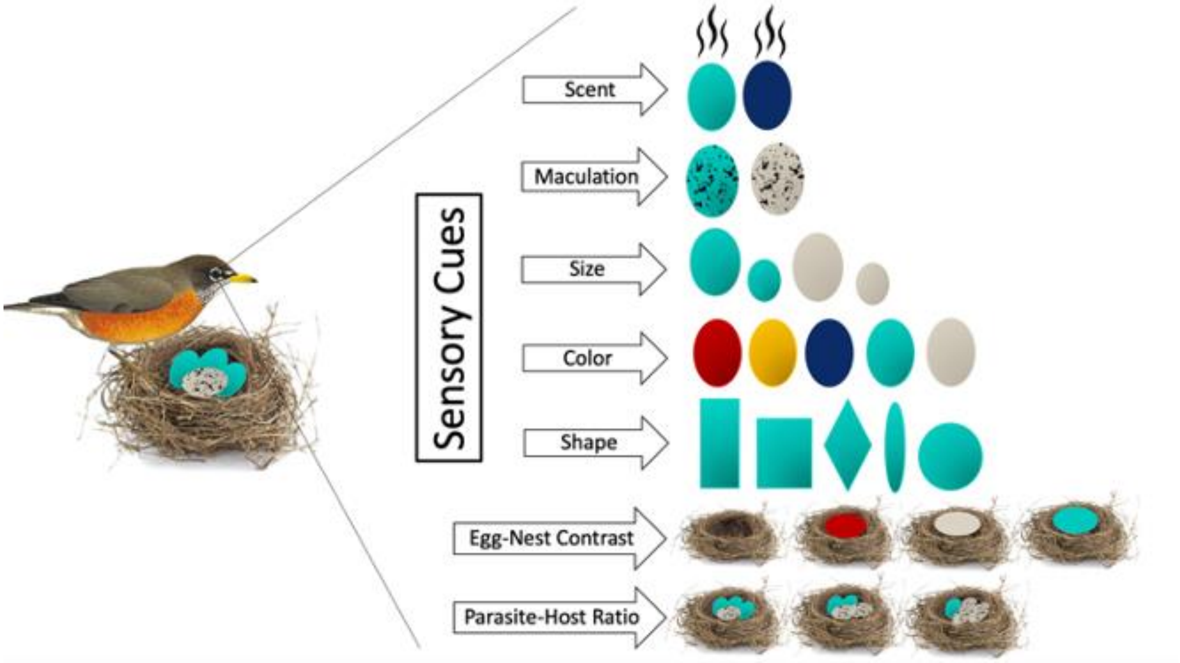

Figure 1.2. Graphic depicting select sensory cues tested in American robins in the context of foreign egg rejection.

TABLE

Table 1.1. Table summarizing the traits analyzed in the published literature and their effect on egg rejection in American robins. N/A columns indicate lack of sample size information to calculate odds ratios. Odds ratios of ∞ indicate values of zero in the denominator of the formula (see main text).

Trait Experimentally Manipulated?	Did Trait Affect Egg Rejection?	Treatment & Control Sample Sizes	Rejection Rate (%)	Odds Ratio (OR)	Source
Classification: Egg Specific Sensory Traits					
Ground Color					
No	Yes	N/A	N/A	N/A	Friedmann 1929
Yes	Yes	N/A	N/A	N/A	Rothstein 1982
Yes	Yes	Cowbird Ground (n=10), Yellow (n=13), Red (n=14), Blue (n=15), Robin Ground Control (n=7)	Cowbird Ground (100%), Yellow (69%), Red (64%), Blue (60%), Robin Ground Control (0%)	∞, ∞, ∞, ∞	Croston & Hauber 2014b
Yes	No	N/A	N/A	N/A	Croston & Hauber 2014a
Yes	Yes	Spotted Parasite (n=11), Immaculate Parasite (n=10), Mimetic Control (n=7)	Spotted Parasite (91%), Immaculate Parasite (0%), Mimetic Control (0%)	∞, 0	Dinets et al. 2015
Yes	Yes	Beige (n=14), Control (n=14)	Beige (79%), Control (0%)	∞	Igic et al. 2015
Yes	Yes	N/A	N/A	N/A	Carmody et al. 2016
Yes	Yes	N/A	N/A	N/A	Hanley et al. 2017
Yes	Yes	Beige (n=19), Robin Mimetic Control (n=19)	Beige (37%), Robin Mimetic Control (0%)	∞	Luro et al. 2018
Yes	Yes	Beige (n=18), Thermochromic (n=14), Control (n=15)	Beige (78%), Thermochromic (43%), Control (7%)	49, 10.5	Hauber et al. 2019

Table 1.1. Continued

Ground Color (UV Reflectance)					
Yes	No	UV-blocked (n=31), Control (n=14)	UV-blocked (10%), Control (7%)	1.39	Abernathy & Peer 2015
Yes	No	UV-blocked (n=5), Robin Ground Control (n=7)	UV-blocked (20%), Robin Ground Control (0%)	∞	Croston & Hauber 2014b
Ground Color (Intraclutch Variation)					
Yes	No	Decreased Variation (n=8), Increased Variation (n=12), Control (n=28)	Decreased Variation (50%), Increased Variation (58.3%), Control (67.9%)	0.47, 0.66	Croston & Hauber 2015b
Ground Color (Blunt Pole Hypothesis)					
Yes	Yes	Deep-Blue on Sharp Pole (n=19), Deep-Blue on Blunt Pole (n=19), Beige Spotted on Sharp Pole (n=13), Beige Spotted on Blunt Pole (n=11), Robin-Mimetic Control (n=10)	Deep-Blue on Sharp Pole (47%), Deep-Blue on Blunt Pole (47%), Beige Spotted on Sharp Pole (85%), Beige Spotted on Blunt Pole (64%)	8.1, 8.1, 49.5, 15.75	Hauber et al. 2021a
Ground Color (Self-Referencing Hypothesis)					
Yes	No	N/A	N/A	N/A	Hauber et al. 2020b
Maculation					
Yes	Yes	Robin Ground & Maculation (n=6), Cowbird Maculation Control (n=18)	Robin Ground & Maculation (83%), Cowbird Maculation Control (94%)	0.29	Rothstein 1982
Yes	Yes	Spotted Parasite (n=11), Mimetic Control (n=7)	Spotted Parasite (91%), Mimetic Control (0%)	∞	Dinets et al. 2015

Table 1.1. Continued

Yes	Yes	Low Elevation: Spotted (n=9), Immaculate (Robin Mimetic, n=6) High Elevation: Spotted (n=9), Immaculate (Robin Mimetic, n=9)	Low Elevation: Spotted (78%), Immaculate (0%) High Elevation: Spotted (22%), Immaculate (0%)	∞, ∞	Carmody et al. 2016
Yes	Yes	Beige Spotted (n=42), Beige (n=19), Robin Mimetic Spotted (n=22), Robin Mimetic (n=19)	Beige Spotted (86%), Beige (37%), Robin Mimetic Spotted (68%), Robin Mimetic (0%)	∞, ∞, ∞	Luro et al. 2018
Yes	Yes	N/A	N/A	N/A	Dainson et al. 2017
Egg Shape					
Yes	Yes	Pointed (n=14), Rounded (n=15), Sphere (n=14), Cylinder (n=14), Rectangle (n=14), Cube (n=14), Egg-Shaped (n=43), Odd-Shaped (n=42), Control (n=13)	Pointed (50%), Rounded (33%), Sphere (29%), Cylinder (79%), Rectangle (57%), Cube (43%), Egg-Shaped (37%), Odd-Shaped (60%)	3.33, 3.33, 1.33, 12.22, 4.44, 2.5, 1.98, 4.9	Underwood & Sealy 2005
Yes	No	N/A	N/A	N/A	Igic et al. 2015
Yes	No	Blue Cowbird Egg Treatment (n=35), Blue Douglas Fir Treatment (n=32), Color Control (n=5), Sanitation Control (n=5)	Blue Cowbird Egg Treatment (65%), Blue Douglas Fir Treatment (97%), Color Control (N/A), Sanitation Control (N/A)	N/A	Luro & Hauber 2017
Yes	Yes	N/A	N/A	N/A	Hauber et al. 2020b

Table 1.1. Continued

Egg Size					
Yes	No	N/A	N/A	N/A	Friedmann 1929
Yes	Yes	Large Cowbird (n=18), Small Cowbird Control (n=11)	Large Cowbird (94%), Small Cowbird Control (100%)	0	Rothstein 1982
Yes	No	N/A	N/A	N/A	Igic et al. 2015
Yes	No	Low Elevation: Cowbird Sized (n=7), Robin Sized Control (n=8) High Elevation: Cowbird Sized (n=7), Robin Sized Control (n=8)	Low Elevation: Cowbird Sized (71%), Robin Sized Control (25%) High Elevation: Cowbird Sized (29%), Robin Sized Control (0%)	7.5; ∞	Carmody et al. 2016
Egg-Nest Contrast					
Yes	No	Beige Nest (n=15), Red Nest (n=15), Robin Mimetic Nest (n=15), Natural Control (n=15)	Beige Nest (0%), Red Nest (30%), Robin Mimetic Nest (25%), Natural Control (0%)	0, ∞, ∞	Aidala et al. 2015
Spatial Chromatic Contrast					
Yes	Yes	N/A	N/A	N/A	Luro et al. 2020
3D Model Egg Material					
Yes	No	Plaster (n=22), 3D Printed (n=28)	N/A	N/A	Igic et al. 2015
Classification: Clutch Composition					
Clutch Size					
No	Yes	N/A	N/A	N/A	Hauber et al. 2019
No	Yes	N/A	N/A	N/A	Abolins-Abols & Hauber 2020a
Parasite-Host Egg Ratio					
Yes	Yes	N/A	N/A	N/A	Rothstein 1975b

Table 1.1. Continued

Yes	No	Majority Robin (n=22), Majority Cowbird (n=25), Equal (n=11)	Majority Robin (86.4%), Majority Cowbird (92%), Equal (82%)	1.4; 2.6	Lang et al. 2014
Olfaction					
Yes	No	Mimetic Citrus (n=14), Non-Mimetic Citrus (n=14), Cowbird Lavage Mimetic (n=13), Non-Mimetic Control (n=10), Mimetic Control (n=10)	N/A	N/A	Hauber 2020
Classification: Maternal Traits					
Maternal Investment in the Egg(s)					
No	No	N/A	N/A	N/A	Hauber et al. 2020a
Maternal Physiology					
No	No	N/A	N/A	N/A	Abolins-Abols & Hauber 2020a
Yes	Yes	Mitotane Treated (n=20), Control (n=17)	Mitotane Treated (75%), Control (41%)	2.1	Abolins-Abols & Hauber 2020b
Body Mass					
No	Yes	N/A	N/A	N/A	Abolins-Abols & Hauber 2020a
Female Age					
No	No	N/A	N/A	N/A	Abolins-Abols & Hauber 2020a
Timing of Parasitism					
Yes	Yes	N/A	N/A	N/A	Abolins-Abols &

Table 1.1. Continued

					Hauber 2020a
Method of Rejection					
No	Yes	N/A	N/A	N/A	Rasmussen et al. 2019
Lateralization					
No	Yes	N/A	N/A	N/A	Scharf et al. 2019
Classification: Spatial Overlap					
Sympatry/Allopatry with Cowbirds					
No	Yes	Cowbird Egg in Allopatric Nests (n=18), Cowbird Egg in Sympatric Nests (Control, n=24)	Cowbird Egg in Allopatric Nests (67%), Cowbird Egg in Sympatric Nests (96%)	0.087	Briskie et al. 1992
Yes	Yes	Cowbird Egg in High Elevation (n=16), Cowbird Egg in Low Elevation (Control, n=16)	Cowbird Egg in High Elevation (75%), Cowbird Egg in Low Elevation (25%)	0.11	Carmody et al. 2016

CHAPTER 2: EGGSHELL TEXTURE BUT NOT ODOR TREATMENT AFFECTS MODEL EGG REJECTION IN AMERICAN ROBINS (*TURDUS MIGRATORIUS)*

ABSTRACT

To curb fitness costs associated with obligate avian brood parasitism, some hosts have evolved to reject foreign eggs in the nest. American robins (*Turdus migratorius)* are among the few hosts of the brown-headed cowbird (*Molothrus ater)* that effectively remove parasitic eggs from their nests. With the parasite's eggs looking nothing like their own, American robins likely rely most on visual cues when making rejection decisions. However, we still know little about the roles tactile and olfactory cues play in robin's or other rejecter hosts' rejection decisions. Here, we conducted a set of experiments to test for the use of tactile or olfactory cues in egg rejection by robins. For the tactile experiment, we found that robins were more likely to reject rough rather than smooth eggs. However, our tactile model egg design was not able to fully discriminate between tactile and visual modalities. For the olfaction experiment, we did not find a significant effect of egg scent treatment on rejection rates. Accordingly, future studies on egg rejection should attempt to fully distinguish between tactile and visual cues, as well as examine olfactory cues in other egg rejecter host species.

INTRODUCTION

Obligate avian brood parasites often impose severe fitness costs on the reproductive outputs of their hosts (Rothstein 1975a). To curb these costs, diverse host species of brood parasitic birds have evolved to reject foreign eggs in the nest (Winfree 1999). Different sensory modalities (e.g., visual, tactile, olfactory) of a parasitic or an experimental model egg's phenotype may be used by hosts of brood parasites to cue egg recognition and/or rejection.

According to a recent meta-analysis (Samaš et al. 2021), visual cues (most importantly, coloration, but also maculation, size, and shape) play important roles in informing egg rejection decisions across hosts of different brood parasitic avian lineages (also see: Turner & Hauber 2021). However, these recent summaries also reveal that we know far less about whether and how tactile and olfactory cues affect egg rejection decisions.

In a limited set of prior experiments, researchers found that tactile cues (in the absence of visual cues) could cause egg rejection (i.e., smaller model parasite eggs were rejected more in the enclosed and lightless nest of one host species, the rufus hornero (*Furnarius rufus;* Mason & Rothstein 1986, Tosi-German et al. 2020). Similarly, only a handful of published studies have experimentally tested for the use of olfactory cue(s) for egg rejection amongst hosts of avian brood parasites, with results providing mixed support (Soler et al. 2014, Hauber 2020).

The American robin (*Turdus migratorius*, hereafter: robin) is a robust rejecter of parasitic brown-headed cowbird (*Molothrus ater,* hereafter: cowbird) eggs by grasping and removing the parasitic egg from the nest (Rothstein 1982; Rasmussen et al. 2009). Therefore, robins are ideal candidates for examining the sensory and cognitive mechanisms involved in accurately identifying a foreign egg and successfully rejecting it from the nest (reviewed by Turner & Hauber 2021). Specifically, robins use several egg characteristics as rejection cues, including egg shape (Hauber et al. 2021b) and size (Luro et al. 2018). These egg characteristics can be perceived as tactile and/or visual cues for egg rejection (Turner & Hauber 2021). Egg shape, especially shapes that increasingly differ from a natural ovoid shape, have higher rates of rejection (Hauber et al. 2021b, Underwood & Sealy 2006), whereas decreasing egg size is a weaker but still consistent predictor of greater rejection rates (Igic et al. 2015, Rothstein 1982);

accordingly, cowbird-sized (i.e., smaller) model eggs are more likely to be rejected than robin-sized (larger) model eggs (Carmody et al. 2016, Luro et al. 2018).

Here, we conducted a new set of experiments to test for the use of tactile or scent cues in the rejection of foreign eggs by American robins, an already well-studied host of the brown-headed cowbird (Turner & Hauber 2021). Specifically, we predicted that (i) superficially rough (sandpaper covered) model eggs would elicit higher egg rejection rates than smooth (cardstock covered as control) model eggs and (ii) scent treatments (peanut oil vs. cowbird cloacal lavage with paraffin oil vs. unscented paraffin oil as control) would not affect egg rejection rates.

METHODS

During April-June 2021, we located active robin nests in parts of Champaign County, IL, USA. Nests were deemed active when (i) the clutch size increased on consecutive days, (ii) the female was flushed from the nest, and/or (iii) eggs in the nest were warm to human touch. We began experimentally parasitizing nests on the first day after clutch completion (i.e., no change in clutch size after two subsequent daily visits), following confirmation that incubation had commenced. For experimental parasitism, we randomly parasitized nests with robin-sized (tactile) or cowbird-sized (scent) 3D printed model eggs, each of which was modelled after a digital image of a natural robin or cowbird egg from the University of California's Berkeley Museum of Vertebrate Zoology's egg collection as a template (for details, see Igic et al. 2015). All nests were experimentally parasitized in the morning hours of the day (i.e., first third of local daylight hours).

For the tactile treatment, smooth (control) model eggs were made using cut strips of Astrobrights™ white cardstock that were hot glued to a 3D printed egg. Sandpaper (rough treatment) model eggs were made using cut strips of Gator premium 60 grit sandpaper that were hot glued to a 3D printed egg. The average height of the grooves of the 60 grit sandpaper was 0.258mm (range: 0.157-0.369mm) when measured with a digital micrometer. Both robin-sized smooth and rough model eggs were painted robin mimetic blue (Figure 2.1), which is ~2 just noticeable difference units (JND) from natural robin egg colors (*sensu* Hauber et al. 2019), in triplicate coats (also see Hauber et al. 2020b). As a positive control for egg rejection, a subset of smooth eggs was painted beige (from Canniff et al. 2018), which is ~5 JND from natural robin eggs (Hauber et al. 2019). Previous research showed that robin-mimetic cowbird-sized model eggs are mostly accepted (>80%) and beige eggs are mostly rejected (>80%) within one day of deployment (Hauber et al. 2020b).

Cowbird-sized model eggs were used for the scent treatments (to parallel Hauber 2020b) and were painted with either a beige color (for positive control; see below) or a non-mimetic deep-blue egg color (Figure 2.2), the latter of which is known to cause ~50% egg rejection within one day of insertion when unscented (Hauber 2020, Hauber et al. 2020b). The peanut oil for the artificial scent treatment was sourced from Acros Organics and the unscented paraffin oil was sourced from Patterson Medical. Cowbird cloacal lavage samples for the natural scent treatment were collected from two different adult female cowbirds captured in seed-baited walk-in traps during May 2021 (i.e., during the local cowbird breeding season) in Urbana (following Hauber 2020a). The lavage scent was created by mixing the cloacal lavage sample with 1 ml of unscented paraffin oil. Both the peanut oil and the lavage treatments were applied to the deep-blue egg types. As a negative control, we applied the unscented paraffin oil to the deep-blue eggs and, as a positive control, we applied unscented paraffin oil to beige eggs. Our scent experiment both quasi-replicated and expanded upon Hauber's (2020a) olfactory treatments as in that study the deep-blue eggs were treated only with artificial scents vs. control solvent and no beige-painted eggs were deployed as positive controls (see below). Scent treatments were applied to model eggs using a Q-tip until the entire egg surface was saturated with the solvent then eggs were placed immediately into the robin nests. By each field researcher on a given day, only one scent treatment was used across different robin nests to reduce cross-contamination of the scents deployed during the same day.

Previous studies of egg rejection in robins found the modal egg rejection latency to be no more than 24 hours (Hauber et al. 2019). Thus, we checked treated nests for rejection/acceptance of the model eggs 24 hours after their deployment. When accepted, we removed the model egg and added a different treatment egg to the nest with a median of 2 (range: 1-4) treatments per nest. Studies completed elsewhere (Croston & Hauber 2014) and at our current study site (Hauber 2020) reported that order of experimentation did not affect the egg rejection outcomes in robins. Depredated (natural robin eggs broken or missing) nests were removed from analyses. Abandonment is not a response to experimental parasitism in our study species (elsewhere: Croston & Hauber 2014, and at our study site here: Abolins-Abols & Hauber 2020a), therefore, abandoned nests (those with cold eggs for two consecutive days) were also removed from analyses.

We used a generalized mixed model in R 1.4.1 to analyze each treatment set. For the tactile dataset, we built a model with the bivariate outcome (accept or reject) as predicted by treatment (rough or smooth) with nest ID as a random effect for the robin-mimetic blue painted eggs only. For the scent dataset, we built a model with the bivariate outcome (accept or reject) predicted by treatment (paraffin oil, peanut oil, or cowbird cloacal lavage) with nest ID as a random effect for the deep-blue painted eggs only. Beige egg treatments for both studies were used as a positive control to indicate background levels of non-mimetic egg rejection in our study population. In turn, only the treatment and negative control were used in the statistical analyses.

RESULTS

Tactile treatments

We found a significant effect of model egg texture (rough vs. smooth) on the egg rejection rates, whereby rough eggs were rejected more than smooth eggs (χ^2=13.1, df=1, p=0.0003, Figure 2.3). Rough eggs were rejected 33% of the time while smooth (negative control) eggs were rejected 13% of the time; our positive control, beige smooth eggs, had a medium rejection rate of 50% (Figure 2.3).

Scent treatments

The scent treatment did not significantly affect the rejection rates of the deep-blue model egg (χ^2=2.5, df=2, p =0.285, Figure 2.4). Twenty-eight percent of peanut oil scented eggs, 40% of cowbird cloacal lavage eggs, and 25% of unscented paraffin (control) eggs were rejected; our positive control, the beige paraffin eggs, had a high rejection rate of 80% (Figure 2.4).

DISCUSSION

Tactile

We found a significant effect of experimental surface texture on egg rejection rates suggesting that tactile cues could play a role in rejection decisions by robins. In a study examining different dimensions of model shapes, another possible tactile cue for rejection, Underwood and Sealy (2006) found that odd-shaped objects (cylindrical and cuboid shaped model eggs) were rejected at higher rates by robins compared to egg-shaped objects (also see Hauber et al. 2021b). Sharp and broken eggshells in the nest are nearly always removed by nesting birds and may also present possible tactile cues for eggshell rejection responses (Kemal & Rothstein 1988, Hauber 2003a). In all these studies, there was a distinct edge present on the eggshells, which can serve as a tactile cue for an incubating female through physical contact with her brood patch.

In contrast, our study modified the texture of the egg as a possible tactile cue rather than altering egg shape as the studies mentioned above. However, the distinction between tactile and visual cues in the context of egg rejection is a difficult one to make in the presence of suitable lighting. Undoubtedly, there is always a visual element suitable for cueing recognition when using unnatural model shapes, textures, or broken eggshells. Our experiment, thus, is not an exclusive test of tactile-only cues as there is likely a visual element as the rougher surface of the sandpaper strips may be perceived visually by robins as different than the cardstock control's smooth surface (see Figure 2.1), even though the visual acuity of robins is much poorer than that of humans (Luro et al. 2020). Therefore, it remains unclear whether the higher amount of egg rejection of the rough eggs was due to strictly tactile, strictly visual, or a combination of both modalities.

Scent

We did not find statistical support for the use of olfactory cues used in robin egg rejection decisions. Similarly, Hauber (2020a) did not find a significant effect of natural (cowbird cloacal lavage), different artificial (citrus) scents, or human handling on the rejection rates of mimetic robin-blue or deep blue cowbird-sized model eggs by robins. These results suggest that this species relies more on other sensory cues when rejecting foreign eggs. However, unnatural scents, such as human handling and tobacco scent, had elicited significant rejection responses in European magpies (*Pica pica),* a host of the great spotted cuckoo (*Clamator glandarius)* (Soler et al. 2014), suggesting that olfactory cueing of egg rejection can play a role in other host-parasite systems, although, even in that study, egg rejection rates were not elevated when natural cuckoo scents were sourced from cloacal lavage or the uropygial gland in experiments.

It is possible that the scents applied to our model eggs did not stay on the egg for long enough or in sufficient concentrations for the female to detect a difference in scents between her own and the model eggs. Future studies in testing the use of olfaction for egg rejection may consider a way for the scent to diffuse from the egg for a longer time. Also, robins build open cup nests and lay larger blue eggs that are significantly different in appearance than those of the brown-headed cowbird which are smaller and beige in ground coloration with brown maculation. Therefore, robins likely rely mostly on visual cues when rejecting foreign eggs, as do most other egg rejecter hosts of brood parasites (Samaš et al. 2021). The use of olfaction, along with other cues, for egg rejection may be instead utilized by host species whose eggs more closely resemble those of the cowbird eggs (Rutledge et al. 2021, Turner & Hauber 2021). Alternatively, olfaction could also aid in individual and/or conspecific egg recognition (Golüke et al. 2016). For example, zebra finches (*Taeniopygia guttata),* a species where intraspecific brood parasitism is common (Shaw et al. 2014), can recognize their own eggs shortly before hatching; however, this species does not reject the conspecific egg (Golüke et al. 2016).

CONCLUSIONS

We found that rough over smooth egg surface texture significantly increased robin egg rejection rates. However, we may not have been able to fully distinguish between tactile and visual modalities of recognition in this robin-focused experiment. Like previous findings in robins, we did not find statistical support for the use of olfactory cues in egg rejection in this species. Due to key visually perceivable differences in egg characteristics compared to their parasite, the brown-headed cowbird, American robins likely rely most on visual cues such as egg color and maculation. Future studies should aim to (a) better distinguish between tactile and visual traits as cues for robin egg rejection and (b) examine all cues discussed in the robin literature in other cowbird host lineages that may have different egg colors and patterns, nest types, and life histories.

ACKNOWLEDGEMENTS

For access to robin nests, we thank the owners of Wandell’s and Country Arbors Tree Nurseries in Urbana, IL, USA. J. Enos assisted with trapping the live adult female cowbirds for us. Funding for this research was provided by a Graduate College Master’s Fellowship and its Travel Grant programs, an Odum-Kendeigh Summer Research Award, and the Francis M. and Harlie M. Clark Research Support Grant in Integrative Biology from the University of Illinois at Urbana-Champaign (to AMT). Additional support was provided by the Center for Advanced Study at the University of Illinois and the Wissenschaftskolleg zu Berlin, Germany (to MEH). For assistance and/or discussions we thank M. Abolins-Abols, E. and F. Hoover, D. Hanley, A. Luro, W. Schelsky, and S. Winnicki.

FIGURES

Figure 2.1. (A) Rough (sandpaper, top) model egg in a natural robin nest on a nursery tree. (B) Smooth (cardstock, bottom) egg in a natural robin nest (situated on the ground, a rare but consistent occurrence at our study site).

Figure 2.2. Deep blue egg (right most) in a natural robin nest on a nursery shrub, immediately after experimental scent application on the model egg.

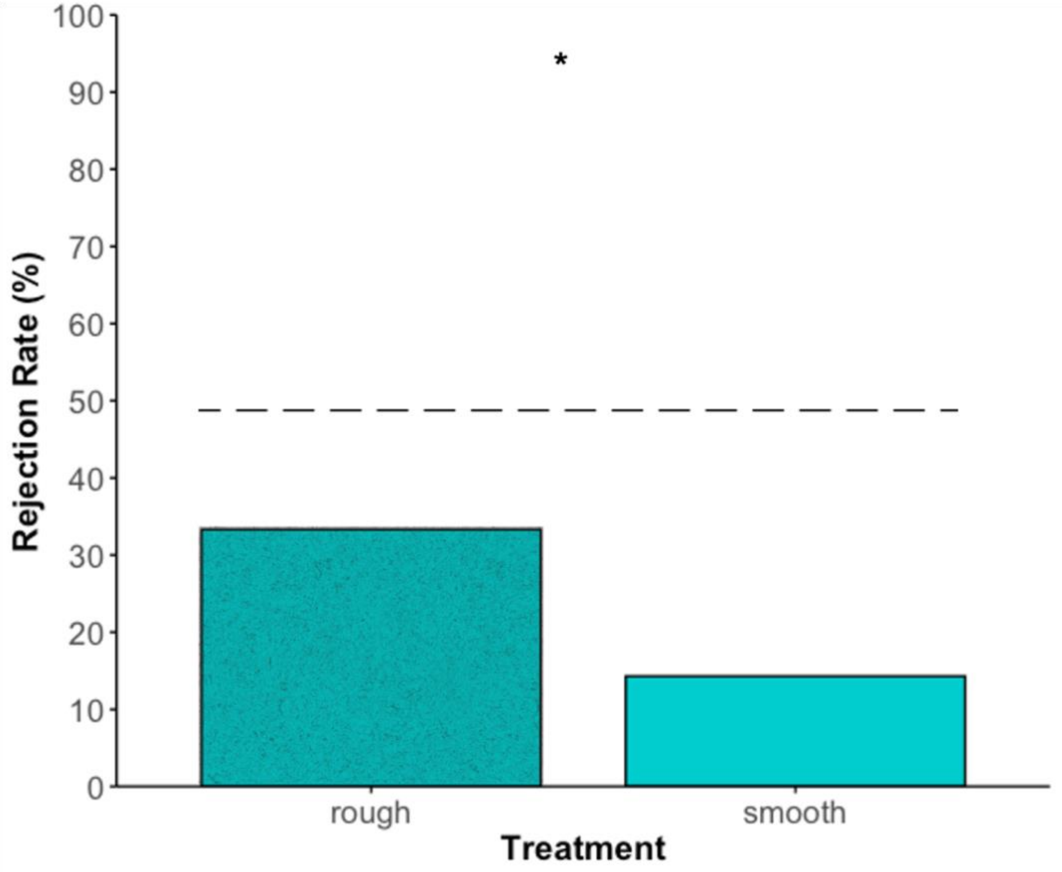

Figure 2.3. Rejection rates of rough (n = 15) or smooth (n = 14) mimetic-colored robin-sized model eggs. The dotted line indicates the rejection rate of smooth beige robin-sized model eggs (n = 10) used as a positive control; the star depicts a significant treatment difference.

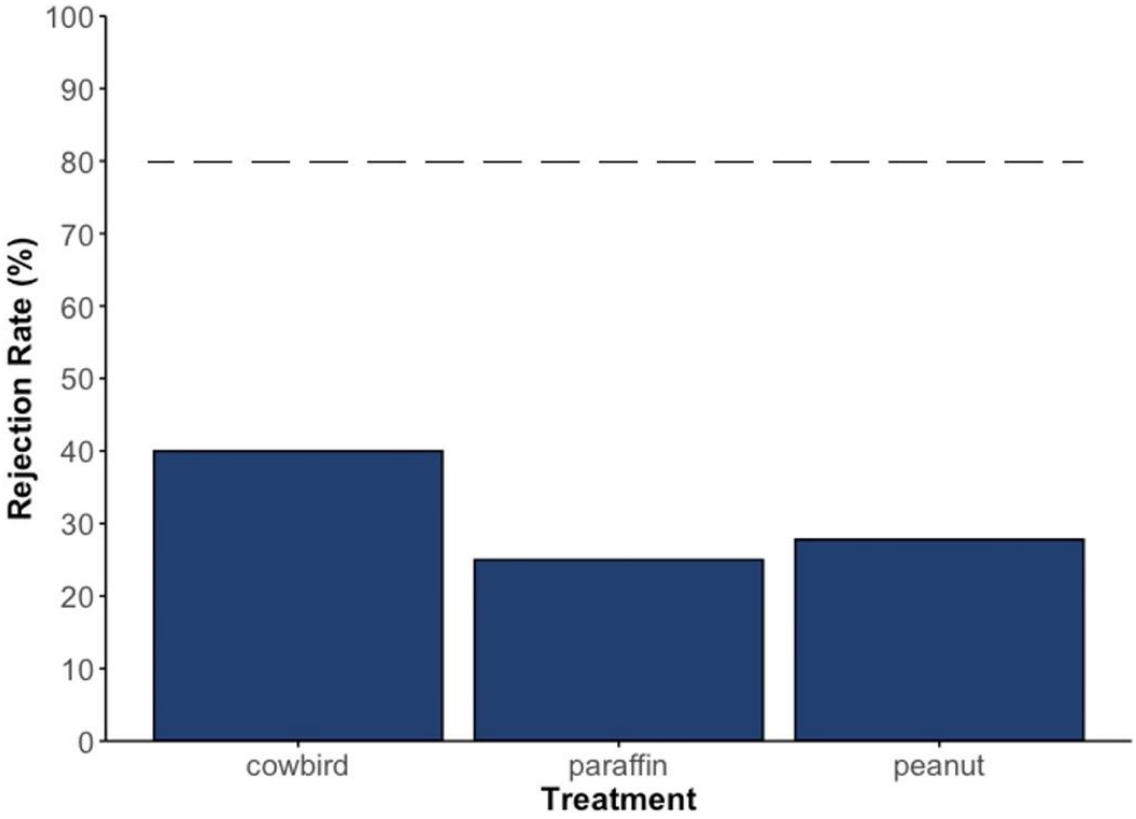

Figure 2.4. Rejection rates of deep-blue cowbird-sized model eggs with cowbird cloacal lavage (n =15), unscented paraffin (negative control; n =16), or peanut oil (n =18) treatments. The dotted line indicates the rejection rate of beige colored model eggs treated with unscented paraffin oil (n =15) as a positive control.

CHAPTER 3: EXAMINING CAUSES AND CHARACTERISTICS OF EGG EJECTION BEHAVIOR IN THE AMERICAN ROBIN (*TURDUS MIGRATORIUS)*

ABSTRACT

American robins (*Turdus migratorius)* are among few hosts of parasitic brown-headed cowbirds (*Molothrus ater)* that frequently eject foreign eggs from their nests. However, we know little about some characteristics of the robins' egg ejection process including the distance and direction taken. We used a novel technique (i.e., radio transmitters inserted into 3D-printed eggshells) to examine egg rejection in female robins as a function of model egg coloration (i.e., robin-mimetic blue, non-mimetic deep blue, and non-mimetic beige). Based on prior work, we predicted that female robins' egg ejection decisions would be dependent on egg coloration. Accordingly, we found a significant effect of female identity and model egg color on egg ejection, but neither predicted ejection distance or direction. Deep blue model eggs had shorter ejection latencies than beige eggs. Fully characterizing the egg ejection process will allow us to further understand the mechanisms and outcomes of egg ejection behaviors in host-parasite interactions.

INTRODUCTION

Individuals in many avian species remove objects from their nests such as debris, broken eggshells, and nestling excrement (reviewed in Guigueno & Sealy 2012). This behavior, known as nest sanitation, promotes the health of offspring by decreasing contamination, protecting the eggs or chicks from egg capping or sharp edges, and hiding the nest from predator detection (Blair & Tucker 1941; Petit et al. 1989; Hauber 2003a). Although most birds engage in nest sanitation behaviors (Tinbergen et al. 1962; Alvarez et al. 1976; Moskát et al. 2003; Underwood & Sealy 2006; Guigueno & Sealy 2009; Hauber et al. 2021b), some are also capable of removing foreign eggs that are laid in their nests by brood parasites (Davies & Brooke 1988; Rothstein 1982; Luro et al. 2018). Egg rejection can be accomplished in many ways and involves the successful recognition of a foreign egg that results in the host's decision to discard or neglect the parasitic egg or the entire parasitized clutch (e.g., Strausberger & Hauber 2017). Ejection is a type of egg rejection behavior in which the host physically removes the egg from the nest. Egg ejection is an effective and adaptive antiparasitic defense behavior to reduce the costs of brood parasitism and is performed by diverse lineages of brood parasite hosts in response to varied sensory cues, including color and maculation (reviewed in Samaš et al. 2021).

However, we know less about where, in which direction, and how far from the nest the rejecter hosts carry and deposit the ejected foreign (parasitic) egg. For example, ejection substrate (as reported by Peer and Sealy 2004b) or distance (as quantified by Sealy & Neudorf 1995) may be relevant for nest success as dropping a foreign egg on solid ground too close to the nest may alert predators of the nest's location. This is because when other biological materials are discarded by parent birds too close to the nest, such as fecal sacs, nests may experience higher predation rates (Petit et al. 1989; Lang et al. 2002 but see Ibáñez-Álamo et al. 2014; Rubio et al. 2018). Egg ejection close to the nest could further or alternatively signal to the brood parasite itself that its egg was rejected from that host nest and result in mafia-like retaliation against the host clutch or brood (Soler et al. 1995; Hoover & Robinson 2007). Ejecting foreign eggs too far from the nest may also incur costs, such as expensive flights or the disruption of host incubation patterns. For example, because parasitism and/or egg rejection often occurs early in incubation (Geltsch et al. 2016), dropping the egg close to the nest may allow the incubating parent to return to the nest rapidly to maintain incubation efficiency and nest protection. Alternatively, ejection far from the nest could encroach on another robin's territory and could potentially be met with an aggressive response from conspecifics (Vanderhoff et al. 2020). Lastly, characteristics such as ejection distance may be representative of the "Goldilocks principle" (Zalasiewicz & Williams 2012) where intermediate distances are more common, driven by both conspecific density and predation risk.

The amount of time from when the host encounters the foreign egg in the nest to its ejection can be referred to as ejection latency and is challenging to measure without direct observations of the nest by researchers or video recorders (e.g., Sealy & Neudorf 1995; Sealy 1996; Peer and Sealy 2004b; Hanley et al. 2015; Scharf et al. 2019). Many factors could potentially affect the amount of time it takes for a host to eject a foreign egg such as host age/past parasitism experience (Martínez et al. 2020), environmental conditions, and egg color (Yang et al. 2022).

The most common and widespread brood parasite in North America is the brown-headed cowbird (*Molothrus ater;* hereafter, cowbird) and it parasitizes well over 200 host species (Lowther 2020). Among these cowbird hosts is the American robin (*Turdus migratorius;* hereafter, robin) that nearly always successfully recognizes and ejects cowbird eggs from its nests (Friedmann 1929; Rothstein 1982). Robin egg rejection and the cues used by this species to make rejection decisions have been studied extensively (reviewed in Turner & Hauber 2021). Here we aimed to re-examine some of the potential factors that influence ejection propensity, as well as newly explore robins' ejection distance, direction, and latency. Previous work has identified visual aspects of the (model) cowbird eggs to be the main factors affecting robin egg rejection (Turner & Hauber 2021); therefore, we presented three different model egg colors in our experiment to the same females in random order. In turn, repeated experiments with the same, non-mimetic deep blue model egg color revealed the repeatability of egg rejection decisions (accept/reject) within the same female subjects (Luro & Hauber 2017) and so we examined the effects of female identity and model egg color on robin's egg ejection behavior. We expected that (i) female identity would predict different aspects of the rejection process and (ii) that model egg color would impact ejection outcome, direction, distance, and latency.

METHODS

3D Printed Model Eggs

We generated plastic model eggs for experiments using a 3D printer (Qidi Tech X-one2). Model eggs were similar in size to cowbird eggs (length: 22mm, width: 18mm) and were painted one of three treatment colors: robin-mimetic blue (*sensu* Canniff et al. 2018), deep blue (*sensu* Luro & Hauber 2017), or beige (quail beige *sensu* Canniff et al. 2018). Model eggs of this beige color are rejected at very high rates in robins (>90%) and are used as an experimental proxy for natural cowbird eggs (rejected over 98%) in many robin egg rejection studies (Hauber et al. 2019; Hauber et. al 2021b). Deep blue model eggs are ejected ~50% of the time by robins and are a crucial color for experimental studies examining what impacts rejection vs. acceptance (Croston & Hauber 2014b; Luro & Hauber 2017; Abolins-Abols & Hauber 2020a,b). Lastly, the paint mix for the robin blue eggs was designed to mimic this host species' own eggs in color (*sensu* Canniff et al. 2018) and have been used by as typically accepted, experimental controls for robin egg ejection experiments (e.g., Igic et al. 2015, Luro et al. 2018; Hauber et al. 2019). Eggs were hollow and printed in halves so that small Pico Pip Ag376 radio transmitter tags from Lotek^{TM} (0.67g) could be inserted into the egg in the field prior to experimental parasitism (see

Figure 2.1). The total mass of the sealed model egg with the radio inside was ~1.54 g, similar to the mass of a natural cowbird egg (3 g, Strausberger 1998).

Before the egg's insertion into a nest, we loosely fed the antenna of the radio transmitter through a small hole on the bottom half of the model egg. Using The Original Super Glue gel^{TM} we glued the top half of the model egg to the bottom securing the transmitter inside the egg. The antenna was 95 mm long and stuck out of the bottom of the egg. After confirming that the glue was dry, we placed the model egg into the nest with the antenna flush with the bottom of the nest (see below).

Experimental Parasitism

Active robin nests were located and monitored during the breeding season from mid-April until early July, 2022 at Wandell's Tree Farm in Champaign County, Urbana, IL, USA (for study site details, see Turner et al. 2022). Once a new nest was found, its GPS location was taken using Google Maps on a cellular telephone (-/+ 1-2 m). Clutch completion was determined using subsequent daily nest inspections and assuming a daily clutch increase of one egg until competition (Rowe & Weatherhead 2009). Within six days of clutch completion, we completed a series of three daily parasitism experiments at each nest, starting with one of the three experimental model egg colors at random. We then watched the nest from a distance of approximately 5 to 10 meters and recorded the time the female returned to incubate (mean=5.8 mins, range: <1 min to >24 mins). We focus on female robins in this study as females are the only parent that incubates in this species (Vanderhoff et al. 2020) and male robins have never been reported to remove parasitic eggs (Sealy & Neudorf 1995; Scharf et al. 2019).

We parasitized a total of 23 different nests that each received all three egg colors in sequential order on subsequent days. However, some data were excluded from measures of ejection latency, distance, and/or direction due to external factors (e.g., weather, lost radio signal, inability to find the model egg, predation). We did not band robins for individual identification in this study, but we conducted all the work during a short period of only three weeks (which is less than a robin's full nesting cycle) and included nesting plot as a random effect in our analyses (see below) to minimize the impact of potential nonindependence by repeatedly parasitizing the same individual female(s). Thus, we used nest identity as a proxy for female identity.

Approximately 24 hours after experimental parasitism, we recorded the rejection status of the first experimental egg. An Automated Radio Telemetry System (ARTS) consisting of one Automated Recording Unit (ARU; JDJC Corp, Fisher, IL, USA), connected to two 3-element Yagi antennas, was used to determine when eggs were rejected. The system was located at the north edge of the study site and had one antenna mounted at 160° and the other at 200° allowing for complete coverage of the study site. The ARTS collected data on the transmitter's signal strength and general location (based on the relative signal strengths received between the two antennas) of the eggs with radio transmitters in them. The location was very coarse and could be only used to assist us in locating the general location of the rejected eggs (e.g., within 50-100 m depending on the distance of nest to the ARTS), which were ultimately located using a hand-held radio receiver (ICOM, Kirkland, WA, USA). The ARTS was programmed for the specific frequencies of the different transmitters allowing for data to be collected from each egg approximately every 15 seconds.

The ARU recorded the data to the second to compare with the exact time at which we artificially parasitized the nest. A stationary egg with a transmitter has a consistent signal strength (dBm), and even if the orientation of the antenna of the egg's transmitter changed in the nest, we would only detect a slight change in the signal strength. In contrast, a signal strength change of more than 10 dBm was used to determine the latency of ejection (the amount of time it took the female to remove the egg from the nest). Accordingly, in many situations we would record small changes (<2 dBm), presumably the robin moving the eggs within the nest, followed by an extremely large change in signal strength (often >30dBm) when the robin had flown with the egg and dropped it away from the nest. This was followed by again an extremely consistent signal strength (changes of <0.5 dBm) as the egg remained on the ground until we retrieved it.

We recorded the GPS location of the nests and the location of the eggs upon retrieval if rejected from the nest. Direction and distance measurements were taken using Google Maps points of the nest and the ejected egg. If the female accepted the model egg, this old egg was taken out of the nest, and a new egg of a different color was added. The same females were experimentally tested with all three egg colors in random order.

Ejection Measurements and Statistical Analyses

For statistical analyses, we used linear mixed effect models (LME) or generalized linear mixed models (GLMM) in R Version 4.1.1 "Kick Things" (R Core Team 2021). Rejection outcome was analyzed using a GLM model with outcome (accept/reject) as the binary response variable and egg color and female identity as fixed effects. For ejection distance, we used an LME model with distance in meters as the response variable and egg color and nest ID as fixed effects with nesting plot as a random effect. Ejection direction was analyzed using circular statistics through the circular (Jammalamadaka & SenGupta 2001) and knitr (Xie 2014) packages in R for a Rayleigh test to determine statistical significance. Ejection latency was analyzed by using simple R scripts to plot change in signal strength. An LME model was used with latency of ejection as the response variable and egg color and nest ID as fixed effects with nesting plot as a random effect. Upon a nest being experimentally parasitized, we recorded when the female returned to the nest and determined when the radio signal strength changed more than 10 dBm (indicated rejection; see above). The ejection latency was the difference in time between when the robin returned and the large change in signal strength.

RESULTS

Rejection Outcome

Rejection outcome was significantly predicted by egg color (χ^2=40.46, df=2, p<0.001) and nest ID (χ^2=58.57, df=23, p<0.001) (Figure 3.1). Robin blue eggs were rejected 30% of the time (7 out of 23), deep blue eggs were rejected in 65% of cases (15 out of 23), and beige eggs had 90% rejection (21 out of 23). In total, 27% of females ejected all three model egg colors, 45% ejected two model egg colors, 18% ejected 1 model egg color only, and 9% accepted all three model eggs.

Ejection Distance & Direction

Neither egg color (χ^2=0.08, df=2, p=0.95) nor nest ID (χ^2=28.06, df=20, p=0.11) significantly predicted ejection distance (Figure 3.2). Ejection distances for all model egg types ranged between 0 and 57.6 meters. Average ejection distance was 28.3 meters (range: 1.2 -57.6 m) for deep blue model eggs, 22.6 meters (range: 0-57.3 m) for beige, and 27.9 meters (range: 0-50.3 m) for robin blue. The direction the ejected egg was taken from the nest was also not significantly different between the different egg colors (Rayleigh's circular test statistic=0.13, p=0.57).

Ejection Latency

The latency of ejection was dependent on egg color (χ^2=9.93, df=2, p=0.006) but not on nest ID (χ^2=18.33, df=18, p=0.43) (Figure 3.3). Deep blue eggs were ejected with shorter latencies than beige eggs (β=216, t=3.151, p<0.01) while there were no significant differences in ejection latencies between beige and robin blue (β=53, t=0.59, p=0.56) and robin blue and deep blue eggs (β=163, t=1.59, p=0.14). Mean ejection times were 11 mins (range: < 1-486 mins) for deep blue, 135 mins (range: < 1-30 mins) for beige, and 130 mins (range: < 1-565 mins) for robin blue eggs.

DISCUSSION

Rejection Outcome

Consistent with our predictions and prior experiments (e.g., Rothstein 1982; Lang et al. 2014; Abernathy & Peer 2015) egg color affected the likelihood of rejection with beige eggs rejected the most, deep blue eggs rejected at intermediate levels, and robin blue eggs rejected the least. These rejection outcomes are also in line with our own previous experiments on egg rejection in robins using the same deep blue (Croston & Hauber 2014b; Luro & Hauber 2017; Abolins-Abols & Hauber 2020a,b) and cowbird-like model egg types (Hauber et al. 2019; Scharf et al. 2019) or the same beige egg color used in this experiment (Hauber et al. 2021b). We found that robin blue eggs had higher (30%) rejection rates than in previous studies (0% in Igic et al. 2015; 0% in Luro et al. 2018; 7% in Hauber et al. 2019); this difference is likely due to the slightly different, more elongated shape and the construction of the model egg (two halves sealed together) relative to cowbird-sized model eggs used before (*sensu* Hauber et al. 2021a). Female identity (through nest identity) was also a significant predictor of rejection outcome suggesting that females that reject one model egg color are more likely to reject another model egg color, too (also see Croston & Hauber 2014a).

Ejection Distance & Direction

Contrary to our predictions, egg ejection distance and direction were not dependent on model egg color or female identity. Prior to this experiment, we anecdotally observed consistent variation in where different females deposited ejected eggs of the different colors. Some females ejected eggs directly below the nest (where we easily found them), while others ejected their model eggs so far that we never found them again or we found them opportunistically on top of the soil in remote parts of the field site with no parasitized nest nearby. Robins are individually repeatable in their decision whether to reject the deep blue egg models within the same breeding attempt (Luro & Hauber 2017); but in this study female robins were tested with different model egg colors in sequence and did not show consistency in their ejection distance and/or direction decisions. Still, a subset of females in our current study clearly showed a preference to drop eggs at certain distances. A third of all females that ejected at least two egg colors that we have distance data for (n=5 out of 15) deposited their model eggs approximately 5 meters from each other and in the same direction. Future studies should focus on measuring individual robins' repeatability of egg ejection characteristics of the same model egg color to determine if individual female robins have consistent differences in the direction, distance, and/or latency of egg ejection.

There are limited experimental studies that have analyzed precise ejection distances in other cowbird hosts. For example, a study on eastern kingbirds (*Tyrannus tyrannus)* documented that kingbirds carry rejected cowbird eggs more than 5 meters from their nests (Bazin 1991, unpublished thesis). Similarly, in northern orioles (*Icterus galbula*), most females deposit cowbird eggs more than 5 meters from their nests (Sealy & Neudorf 1995). Researchers' visual observations have been documented in other rejecter cowbird hosts such as the gray catbird (*Dumetella carolinensis*) and warbling vireo (*Vireo gilvus*) (see Table 2 in Sealy & Neudorf 1995). Most rufous hornero (*Furnarius rufus*) individuals, that are hosts of the shiny cowbird (*Molothrus bonariensis*), rejected model eggs by leaving it in the entrance tunnel of their elaborate domed nests; a subset of these individuals further removed it from the entrance tunnel on their way out of the nest (Tosi-Germán et al. 2020). Only two of these individuals carried the egg out of the nest directly from the brood chamber (Tosi-Germán et al. 2020).

The disposal behavior of nestlings' fecal sacs may provide a parallel scenario similar to foreign eggs removed by parent birds and disposed of outside of the nest. Guigueno and Sealy (2012) demonstrated that when parent birds of varying species remove non-egg biological materials from the nest, such as fecal sacs, the distance that these were carried was directly proportional to adult body mass. Unfortunately, we did not capture our robin females and so are unable to determine if body mass positively correlates with egg ejection distance. In white-breasted nuthatches (*Sitta carolinensis),* fecal sac disposal is non-random, where 95% of fecal sac disposal occurs within their foraging area 6-60 meters from the nest's location (Weitzel 2005). This was also the case for western bluebirds (*Sialia mexicana*), whose direction of sac disposal was concentrated towards foraging sites, 91 meters on average from the nest (Weitzel 2003). The disposal of other biological matter from the nest on the way or within foraging areas would be energetically economical and could potentially be a strategy used by robins when ejecting foreign eggs from their nests.

There may also be energetic costs associated with carrying the foreign egg to farther distances as there are with parents carrying fecal sacs; this can be especially relevant if a host is carrying the larger fecal sacs of the parasitic cowbird nestlings compared to their own young (Guigueno & Sealy 2012). Female robins typically consume or carry fecal sacs of their own nestlings out of the nest (Vanderhoff et al. 2020) and these fecal sacs likely weigh less (~1-3 g, based on Quan et al. 2022) than a cowbird egg (range for a whole fresh egg: 2.63-3.36 g, Lowther 2020). Therefore, it is possible that robins experience a greater energetic cost for ejecting foreign eggs at longer distances.

Ejection Latency

Studies from other rejecter hosts have found a range of ejection latencies for natural cowbird eggs. Warbling vireos ejection latencies range from less than an hour up to 96 hours (Sealy 1996). Northern orioles take anywhere from 1-35 minutes to remove cowbird eggs from their nests (Sealy & Neudorf 1995). Great-tailed (*Quiscalus mexicanus*) and boat-tailed grackles (*Quiscalus major*), that retain their rejection in allopatry with cowbirds, take anywhere from 1-72 hours to eject brown or bronzed cowbird (*Molothrus aeneus*) eggs from their nests (Peer & Sealy 2004b). Eastern bluebirds (*Sialia sialis*) that ejected a cowbird-mimetic model egg did so between 1 and 14 minutes (Butler et al. 2020).

In our study, female robins that ejected the deep blue egg all did so in under an hour while beige and robin blue model eggs had more variable ejection latencies (Figure 3.3). Other studies at our study site have shown wide variation in rejection times in robins for a similar but lighter beige color where 33% were rejected in under an hour (Scharf et al. 2019) and 80% rejected by the second day following the addition of the model egg (Hauber et al. 2019).

The deep blue model egg is at an avian visually-perceptible distance of ~ 19 Just Noticeable Difference units (JND, *sensu* Croston & Hauber 2014b) from the robin's natural egg color (Croston & Hauber 2014b; Hauber et al. 2020b). Thus, it is possible that females are able to rapidly perceive the difference in this deep blue color and hence make a decision quickly. However, this does not explain the nearly half of females that accepted this same model egg color despite the greater chromatic contrast (Hauber et al. 2020b). Nevertheless, the beige color (5 JNDs from the robin's own egg color; Hauber et al. 2021a) also more closely resembles that of their brood parasite's eggshell ground color (4 JNDs; Hauber et al. 2021a), and it is surprising that rejection times for this color varied so broadly. In turn, robin blue (2 JNDs, Croston & Hauber 2014b) rejection times showed a smaller variation in latency than the beige and with most of these eggs accepted overall.

CONCLUSION

Much remains to be discovered about what impacts hosts' egg rejection decisions regarding how fast to eject foreign eggs from the nest and where to drop them, as well as the implications of these decisions for overall reproductive success. We now know that the latency of ejection can be affected by egg color, with deep blue eggs being ejected out of the nest the quickest in our current study. Further, individual female traits associated with egg ejection may influence subsequent nest survival and individual fitness. The factors affecting whether and where the robin may consistently deposit the same egg type (i.e., distance, direction) need further examination using repeated experiments with the same model egg color. It is possible that female age, body mass, and energetic costs could also be important factors for investigating certain characteristics of egg ejection.

ACKNOWLEDGEMENTS

We are grateful to the owners of Wandell's Tree Nursery for allowing us to carry out this study. Support for AMT was further provided by the Edwin Banks Memorial Award from the department of Evolution, Ecology, and Behavior at the University of Illinois at Urbana-Champaign. For discussions we thank S. Lawson and M. Chamberlain.

FIGURES

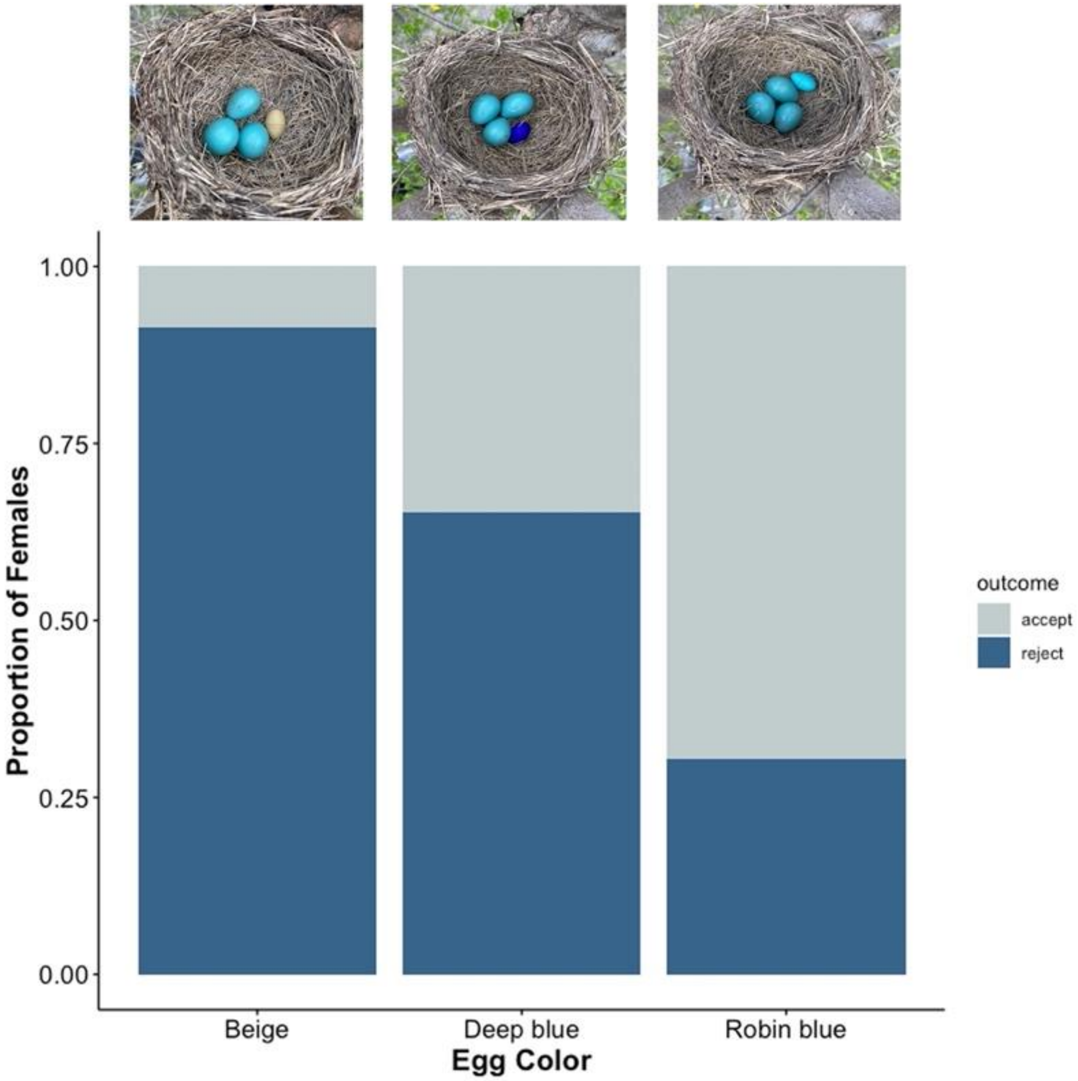

Figure 3.1. Proportion of female American robins that ejected the beige (n=21/23), deep blue (n=15/23), and robin blue (n=7/23) model eggs. Photos of painted model egg types with radio transmitters inserted are shown in experimental robin nests above the respective bars (photo credits: AMT).

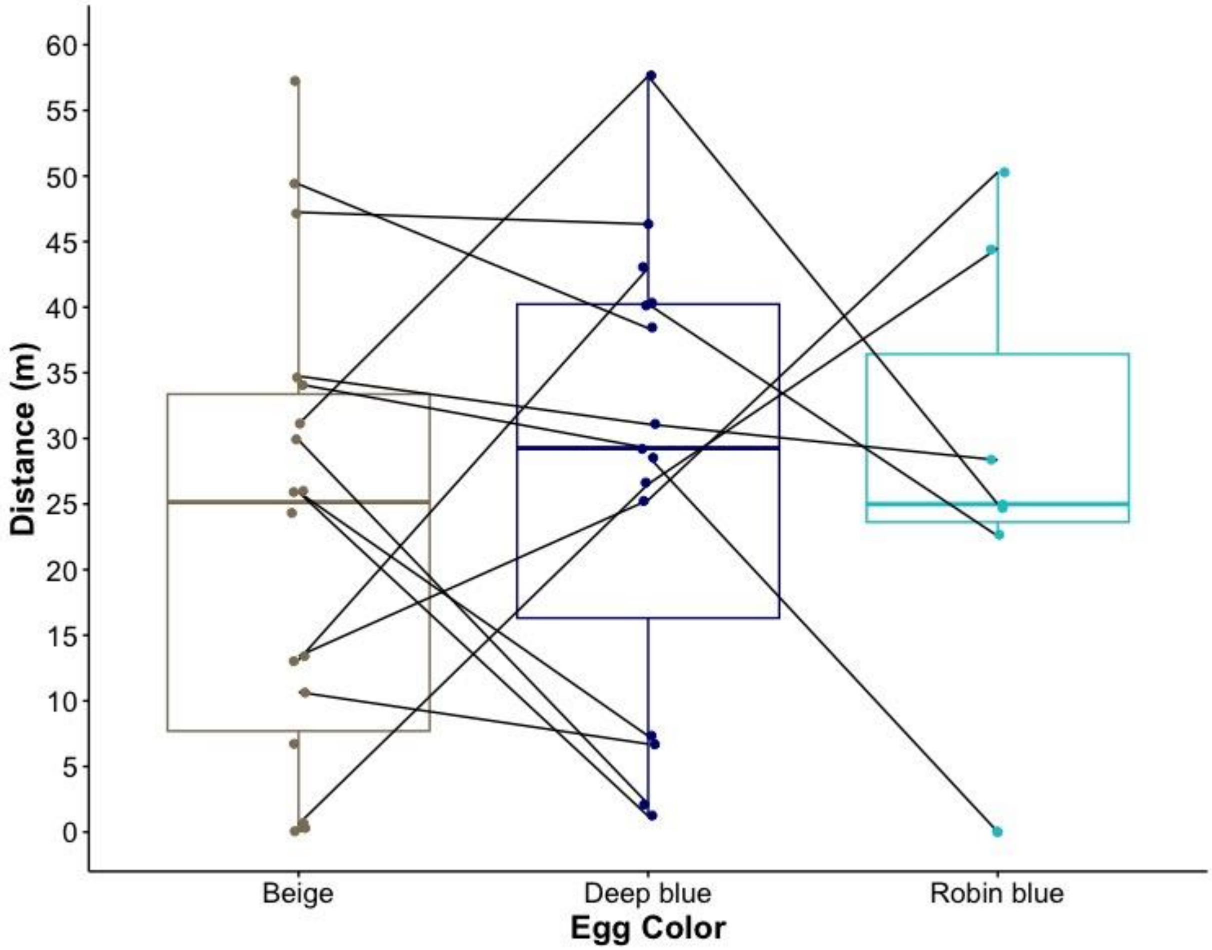

Figure 3.2. Distance of the ejected eggs for beige (n=18), deep blue (n=15), and robin blue (n=7) model eggs. The box plots indicate 10th, 25th, 50th, and 90th percentiles. The lines connect individual females' distances for each model egg color ejected.

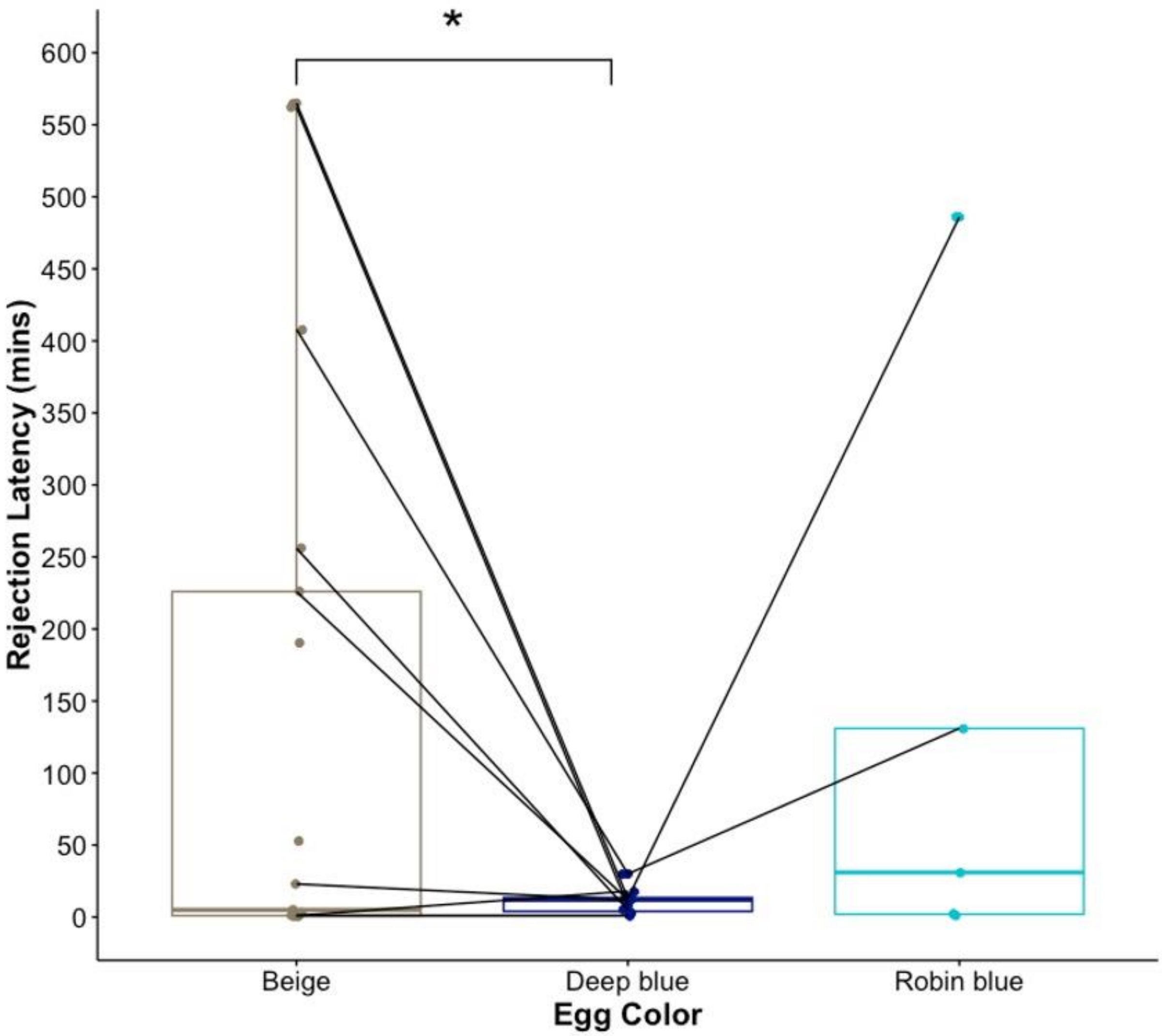

Figure 3.3. Ejection latencies (in minutes) for beige (n=19), deep blue (n=15), and robin blue (n=7) model eggs ejected by female American robins. The asterisk represents statistical significance between beige and deep blue model egg colors. The box plots indicate 10th, 25th, 50th, and 90th percentiles. The lines connect individual females' latency responses for each model egg color ejected.

CHAPTER 4: THE REPEATABILITY OF FEMALE BEHAVIORS DURING THE ANTI-PARASITIC EGG EJECTION PROCESS IN THE AMERICAN ROBIN (*TURDUS MIGRATORIUS)*

ABSTRACT

Egg ejection is an antiparasitic defense behavior in potential hosts that involves the successful recognition and removal of foreign eggs laid by obligate brood parasites. Some hosts are repeatable in their ejection decisions (reject/accept) of the same model egg type, but few studies have examined the repeatability of other traits associated with this behavior (i.e., distance and direction taken, latency of ejection). American robins (*Turdus migratorius)* are robust ejecters of natural and model parasitic brown-headed cowbird (*Molothrus ater*) eggs added to their nests. Here, using radio transmitters inside 3D printed model eggs, we asked if ejection characteristics such as distance, direction, and latency are repeatable in this host. We found that distance and direction were not consistent within individual females whereas ejection latency and female return time were consistent. Further studies are needed to assess how proximate factors that impact ejection characteristics also influence the repeatability of these traits in the antiparasitic responses of diverse host lineages.

INRODUCTION

Despite the fitness costs of raising foreign offspring in their nest (Hauber 2003b), only a handful of the host species of the generalist brood parasitic brown-headed cowbird (*Molothrus ater*; hereafter: cowbird) can successfully recognize and remove parasitic eggs from their nests (Winfree 1999). This behavior, known as egg ejection, is an effective way to decrease the fitness costs associated with parasitism (Samaš et al. 2021). In turn, the repeatability of certain behavioral traits across the animal kingdom is well-documented (Bell 2009); however, the individual repeatability of foreign-egg ejection has been relatively understudied (but see Samaš et al. 2011, Croston & Grim et al. 2014, Hauber 2014, Luro & Hauber 2017).

When studied, hosts appear to be individually repeatable in their ejection decisions of the same egg type both within the brown-headed cowbird-host system (Croston & Hauber 2014a, Luro & Hauber 2017) and in other brood parasite-host systems (Samaš et al. 2011, Grim et al. 2014). For example, in thrushes such as the Eurasian blackbird (*Turdus merula)* and song thrush (*Turdus philomelos),* that are intermediate rejecters of both non-mimetic and mimetic foreign-egg types, individuals are significantly repeatable in their rejection responses (Samaš et al. 2011, Grim et al. 2014). However, this level of repeatability varies in other host-brood parasite systems such as that of the Eurasian magpie (*Pica pica*) and the parasitic great spotted cuckoo (*Clamator glandarius*), with magpie hosts switching their responses from acceptance to rejection as they age across breeding attempts and seasons (Molina-Morales et al. 2014, Martínez et al. 2020).

American robins (*Turdus migratorius;* hereafter: robin) are well known egg ejecters of natural brown-headed cowbird eggs and of other, non-mimetic model egg colors; hence they are often used in experimental antiparasitic egg-ejection studies (reviewed in Turner et al. 2021). Robins are individually repeatable even with intermediately ejected egg colors (Luro & Hauber 2017) and individuals that eject one model egg color are more likely to reject another color (Croston & Hauber 2014a, Turner et al. 2023).

However, we know little about the proximate causes and the repeatability of other aspects of the egg ejection process (i.e., where, and how fast eggs are ejected). Disposing the egg too far from the nest could potentially result in costs through energetically expensive flights while carrying a foreign egg (e.g., as seen in fecal sac disposal, see Discussion below and Guigueno & Sealy 2012) or by disrupting the incubation process. On the other hand, ejection too close to the nest could act as a cue to nest predators. In turn, whether and how quickly eggs are removed (i.e., latency) may depend on individual female's previous experience with foreign eggs (Hauber et al. 2006, Xu et al. 2023). For example, assuming a learned component for egg rejection in experienced females, individuals may respond faster and more accurately with each subsequent parasitism event (Xu et al. 2023). Female age has been shown to affect the likelihood of rejection in other hosts (Lotem et al. 1992, Lotem et al. 1995, Hauber et al. 2006, Molina-Morales et al. 2014, Moskát et al. 2014a, Martínez et al. 2020, see Discussion).

In our previous work, using the novel technique of radio transmitters having been inserted in 3D printed model eggs, we repeatedly parasitized the same female robin with three different model egg colors commonly used in robin ejection experiments (more cowbird-like beige, artificial deep blue, and robin-mimetic blue) to start to understand female ejection characteristics (i.e., distance and direction taken, the latency of ejection) (Turner et al. 2023). Individual females showed much individual variation in their ejection characteristics of the different model egg colors; however, a small number of females appeared to have preferences for the distance and direction model eggs were deposited (Turner et al. 2023). In turn, the latency of ejection was dependent on model egg color (Turner et al. 2023). However, that study was not a suitable assessment of individual repeatability itself since the egg colors used in each subsequent parasitism experiment were different than in the previous ones given.

Here we aim to test statistically for individual repeatability in egg ejection characteristics in female robins using the same, beige model egg color repeatedly at the same nests. We predict that females repeatedly parasitized with a beige model egg will be (i) individually repeatable in their ejection distances, direction, and latency. Alternatively, whether or not the latency of ejection is consistent within individual females, (ii) ejection latency may also decrease with each parasitism event providing evidence for prior experience-dependence in egg rejection traits.

METHODS

Model Eggs

Our model cowbird-egg sized and shaped eggs were 3D printed in two halves to fit a radio transmitter inside. The model eggs used for this experiment were from the same source as used in our previous work (see Turner et al. 2023 for details on model egg design and manufacture). We used a beige color (quail beige *sensu* Canniff et al. 2018) that is ejected by robins at consistently high rates both when containing a radio-transmitter (Turner et al. 2023) or when using it on a solid 3D printed model egg (e.g., Hauber et al. 2019, Hauber et al. 2021a).

Experimental Parasitism

Active onsets of robin nesting attempts (i.e., robins building or laying) were located and monitored during the breeding season from mid-April until early July, 2023 at Wandell's Tree Farm in Champaign County, Urbana, IL, USA. GPS locations of new nests were taken using Google Maps on a cellular telephone (-/+ 1-2 m). Clutch completion was determined using subsequent daily nest inspections and assuming a daily clutch increase of one egg until completion (Rowe & Weatherhead 2009). Within six days of clutch completion (i.e., in the first half of the incubation cycle), we completed a series of three daily parasitism experiments at each nest. Prior work showed no difference in robin's egg rejection rates as a function of time since clutch competition (Abolins-Abols & Hauber 2020b). We then watched the nest from approximately 5 to 10 meters away and recorded the time that a female returned to incubate. Females are the only parent that incubates in this species and are, thus, our only target sex for this study (Vanderhoff et al. 2020).

We parasitized 15 different nests by placing a single beige egg in each nest on 3 consecutive days. We did not band robins for individual identification in this study and used nest identity as a proxy for female identity. We also included nest site plot in our analyses (see below) to minimize the impact of potential nonindependence by repeatedly parasitizing the same individual female(s) (as in Turner et al. 2023).

Approximately 24 hours after experimental parasitism, we record whether the first experimental egg was rejected or accepted. To determine the latency of ejection for each parasitism event, we used an Automated Radio Telemetry System (ARTS) that included one Automated Recording Unit (ARU; JDJC Corp, Fisher, IL, USA) connected to two 3-element Yagi antennas. For specific descriptions of ARTS setup and latency determination using the ARU, please refer to Turner et al. (2023). Eggs that were ejected from the nest were tracked with a handheld receiver (ICOM, Kirkland, WA, USA). We recorded the GPS location of nests and the location of the eggs upon retrieval. This data was used to assess the direction and distance between the nest and the ejected egg. If the female accepted the model egg, this old egg was taken out of the nest on the subsequent daily check, and a new beige egg was added.

Ejection Trait Measurements and Statistical Analyses

For statistical analyses of the repeatability of ejection distance, latency, and female return time, we used the rptR package (Stoffel et al. 2017) in R Version 4.1.1 “Kick Things” (R Core Team 2021). In every rptR model, nest identity (ID) was specified as the grouping variable to estimate the adjusted repeatability for each female and the order the beige egg was given and experimental nesting plot were included as fixed effects. For distance, the original model that included plot did not converge; thus, we report the findings for distance without plot included. We then performed bootstrap and permutation analysis for every rptR model (see Table 1). To test for a learned component for ejection latency and nest return time, we included separate linear models with latency and nest return time as the response variable and the order of the beige egg as a predictor. Ejection direction was analyzed using circular statistics through the circular (Jammalamadaka & SenGupta 2001) and knitr (Xie 2014) packages in R for a Rayleigh test to determine statistical significance.

RESULTS

Distance and Direction

The distance each beige egg was carried was not statistically repeatable within individual females (R=0.11, p=0.25, Figure 1, see Table 1). Average ejection distance was 36.2 meters (range: 0.9-82.6 meters). Similarly, the direction the female carried the egg was also not individually repeatable (Rayleigh's test statistic= 0.23, p=0.12).

Latency

We did not detect a significant difference of the repeatability of ejection latencies in the original model (R=0.66, p=0.19, Figure 2). However, after bootstraps and permutation, we found latency to be repeatable by nest ID (R=0.66, p=0.02, see Table 1). Mean ejection times for all females was 86.7 minutes (range: 1-938 minutes). The order that the beige egg was given was not a significant predictor of ejection latency ($F_{2,31}$=0.78, df=2, p=0.47).

Female Return Time

The time it took the female to return to the nest after she was flushed from the nest for experimental parasitism was individually repeatable (R=0.49, p=0.02, Figure 3, see Table 1). The average return time was 8.9 minutes (range: 2-23 minutes). The order that the beige egg was given was not a significant predictor of nest return time ($F_{2,33}$=1.1, df=2, p=0.36).

DISCUSSION

Ejection Outcome

Female robins were highly consistent in their ejection decisions of the beige model egg. Only one female accepted the beige model egg, and she only accepted it for the first trial as she ejected the same model egg type in her following trials. This consistency in beige-egg rejection is in line with previous studies in robins using the same-colored model egg (Hauber et al. 2019, Hauber et al. 2021a, Turner et al. 2023).

Distance and Direction

Female robins showed no statistical patterns of repeatability in the distance and direction to which model eggs were taken. Females ranged from dropping the model egg right below the nest to flying off with it over 70 meters from their nests (Figure 1).

While we know that robins are individually repeatable in their ejection decisions (reject/accept, Luro & Hauber 2017), where they choose to deposit the egg may be an aspect of this complex behavior that fits an adaptive function to vary. Fecal sac disposal from nestling birds provides a parallel behavior to examine when it comes to analyzing ejection distances/directions. Accordingly, some studies on fecal sac disposal have found that fecal sacs deposited close to the nest can increase predation rates (Petit et al. 1989, Lang et al. 2002 but see Ibáñez-Álamo et al. 2014, Rubio et al. 2018). Thus, an increase in predation rates could be experienced by females that dispose of foreign eggs closer to the nest if a cracked egg on the ground signals to predators the nest location. At the egg stage, robin nests at our site are susceptible to predation from array of avian and ground predators that may notice a cracked egg such as raccoons (*Procyon lotor)*, fox snakes (*Pantherophis ramspotti)*, garter snakes (*Thamnophis sirtalis)*, cowbirds, and common grackles (*Quiscalus quiscula)* (Winnicki et al. 2022). Alternatively, female robins could be depositing the eggs on their way to engage in other activities (e.g., foraging) and the direction they take may vary.

Guigueno and Sealy (2012) found that fecal sac disposal of various species to be proportional to the body mass of the parent. Energetic costs are especially likely if the parent bird is carrying a fecal sac of a larger brood parasite in the nest (Guigueno & Sealy 2012). Since the egg is larger than a fecal sac in both weight and size (see Turner et al. 2023), and if the egg is being carried to farther distances from the nest, then there could be more energetic costs at play for the female robin when ejecting foreign eggs. Additionally, the direction that the female takes the egg may add additional costs if she is not disposing of it while performing other behaviors (e.g., foraging). Western bluebirds (*Sialia mexicana)* dispose of their nestling's fecal sacs in the direction of their foraging areas (Weitzel 2003) which could be an energetically economical strategy. For our study, we did not capture the female robins and were unable to determine if their body mass positively correlated with ejection distance; this would be key information to collect in future studies.

Latency

After accounting for bootstraps and permutation, our results show a statistical pattern towards individual female consistency in how quickly beige model eggs were removed from the nest. About half of the females (n=7) had ejection latencies of a minute or less for all beige eggs ejected. In a different host-parasite system, when given the same model egg type, Eurasian blackbirds are moderately repeatable in their rejection latencies while song thrushes showed low levels of latency repeatability (Samaš et al. 2011). Consistent ejection latencies may be indicative that females have prior experience with removing foreign eggs in their nests. Female age and prior parasitism experience has been shown to affect rejection rates in other host-brood parasite systems (Lotem et al. 1992, Lotem et al. 1995, Hauber et al. 2006, Molina-Morales et al. 2014, Moskát et al. 2014a, Martínez et al. 2020 but see Procházka et al. 2014) but potentially not in the robin (Abolins-Abols 2020b). Other female robins in our study (n=3) decreased their ejection latencies with each additional beige egg providing some evidence for experience-dependence. We did not detect a statistical effect of the order the beige egg was given on ejection latency, however, the number of females that decreased their ejection latencies over time was small with most females showing individual consistency. It is possible that the few birds with decreasing ejection latencies were parasitism-naïve or altogether younger; although, these age-related factors were not assessed or controlled in our study.

It is critical to consider that the latency of foreign-egg ejection may not directly and positively correlate with how quickly the egg is recognized as foreign and/or how quickly the female decides she will remove the egg. Observations from previously recorded experimental parasitism nests that female robins will often inspect the egg before continuing incubation and dispose of the egg later (Scharf et al. 2019). Most females during incubation only leave the nest to forage; thus, it would be interesting to know if some females delay egg removal until their next feeding bout (e.g., Hauber et al. 2019).

Female Nest-Return Time

Females were often flushed off the nest by the researcher to add the model egg. Therefore, we also recorded the amount of time it took the female to return to the nest upon our disturbance to get accurate latency data and, because, in some other *Turdus* species being flushed from the nest positively predicted egg rejection behavior (e.g., Hanley et al 2015). Unpredictably, females were consistent in the time it took them to return to the nest after being disturbed for experimental parasitism. This consistency, especially in shorter return times, likely benefits incubation if females are limited in how much time they can spend off the nest.

This finding regarding nest-return latencies may also speak to the role of females' behavioral personality in antipredator responses. Although, the repeatability of egg ejection behavior may be different depending on the perceived risk of the nest threat, and it is possible that human researchers could be perceived as predators or brood parasites (assuming the female witness the addition of the egg) (e.g., Hanley et al. 2015, Abolins-Abols & Hauber 2020a). We did not record the distance and direction birds flushed during the addition of the egg (known as flight initiation distances: Abolins-Abols & Hauber 2020a) and thus, were unable to determine if the female witnessed the experimental parasitism.

CONCLUSION

Much remains to be discovered about the proximate factors impacting (i) ejection characteristics themselves and (ii) the repeatability (or non-repeatability) of these traits. Future studies should consider the role of the incubating female's age and experience with brood parasitism (*sensu* Xu et al. 2023), the egg removal's energetic costs, and the locale of the egg disposal, and the path to foraging sites. Examining these characteristics in other ejecter hosts will also provide broader insights into the role of behavioral personality traits on host-brood parasite coevolution (*sensu* Avilés & Parejo 2011, Møller & Si 2021, Zhang et al. 2021).

FIGURES

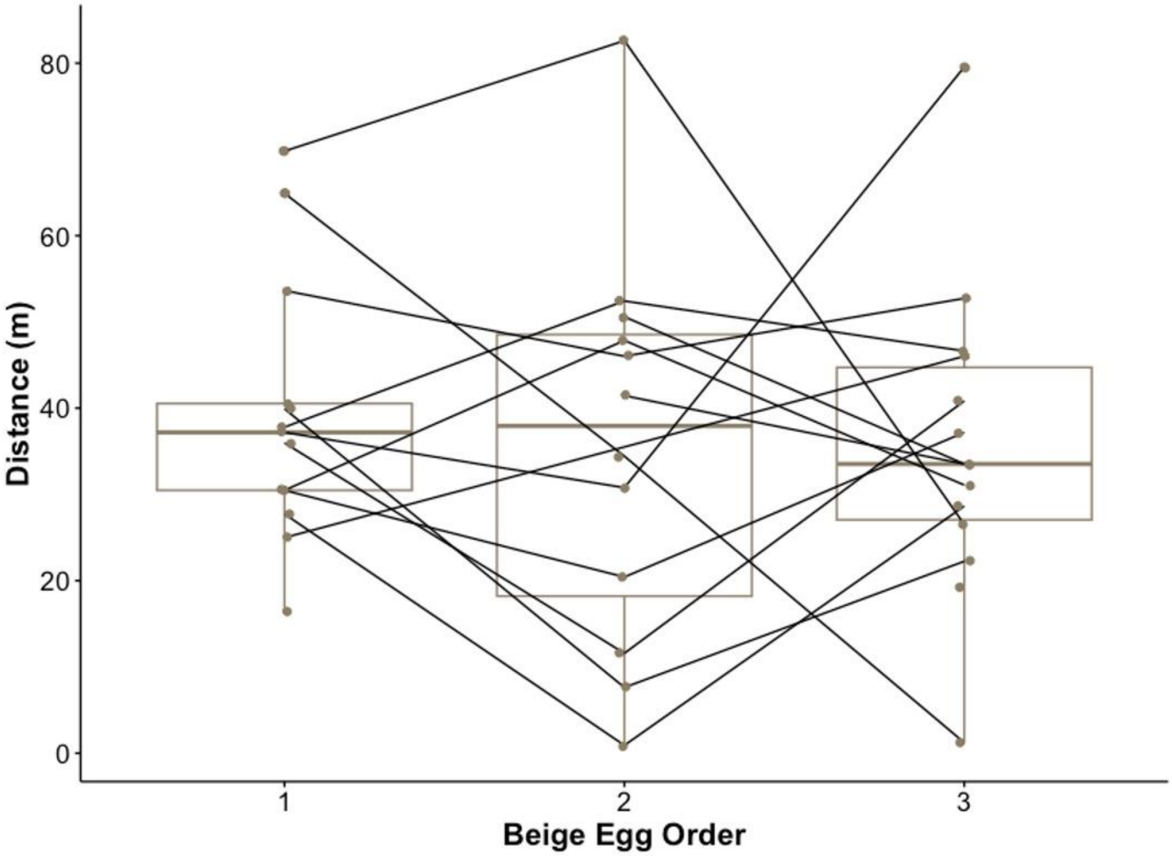

Figure 4.1. The distances of the ejected beige eggs given first (n=13), second (n=14), and third (n=14) at the same nests. The lines connect individual females' distances for each beige egg ejected. The box plots indicate 10th, 25th, 50th, and 90th percentiles.

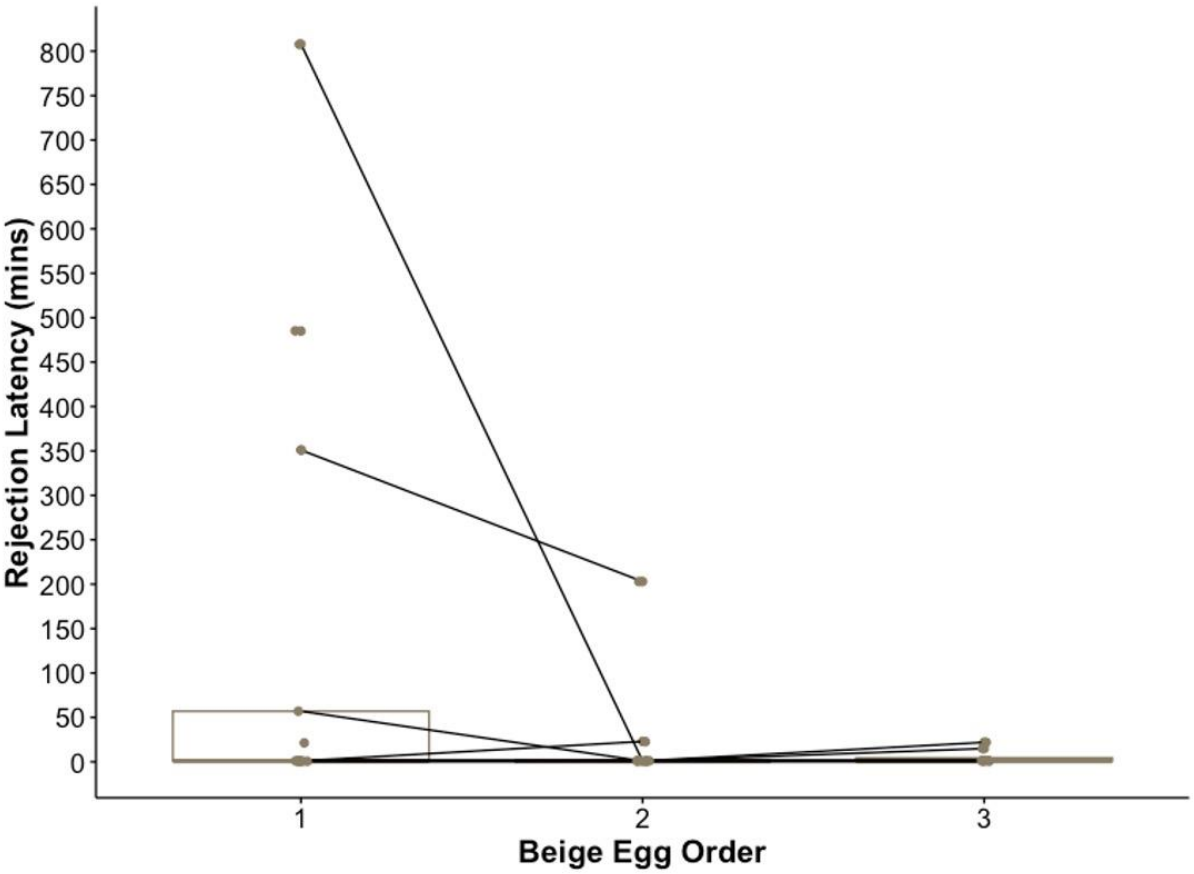

Figure 4.2. Ejection latencies for beige eggs given first (n=13), second (n=13), and third (n=8). The lines connect individual females' latency responses for each beige egg rejected. The box plot indicates 10th, 25th, 50th, and 90th percentiles.

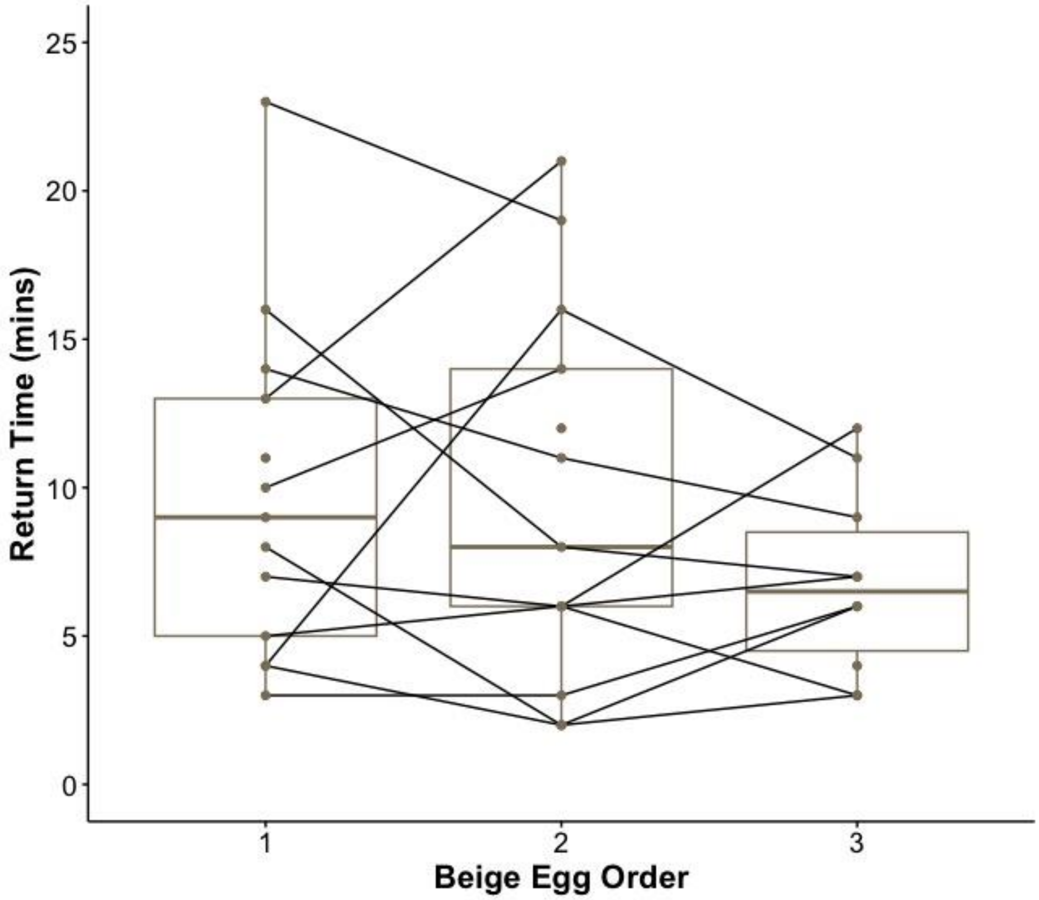

Figure 4.3. Female return times for beige eggs given first (n=13), second (n=13), and third (n=10). The lines connect individual females' latency responses for each beige egg rejected. The box plot indicates 10th, 25th, 50th, and 90th percentiles.

TABLE

Table 4.1. Model outputs with and without bootstraps and permutation for distance, latency, and return time.

Response Variable	Fixed effect + **grouping variable**	Output	
		nboot & npermut=0	**nboot & npermut=1,000**
Distance (m)	beige order + **nest ID**	R=0.11, p=0.25	R=0.11, SE=0.14, CI [0, 0.45], p=0.26
Latency (mins)	beige order + plot + **nest ID**	R=0.66, p=0.19	R=0.66, SE=0.17, CI [0.23, 0.87], p=0.02
Return Time (mins)	beige order + plot + **nest ID**	R=0.49, p=0.02	R=0.49, SE=0.20, CI [0, 0.78], p=0.02

CHAPTER 5: NON-INVASIVE ELEVATION OF CIRCULATING CORTICOSTERONE INCREASES THE REJECTION OF FOREIGN EGS IN FEMALE AMERICAN ROBINS (*TURDUS MIGRATORIUS)*

ABSTRACT

Avian obligate brood parasites rely on other species to raise their offspring. In turn, many brood parasite hosts have evolved defensive behaviors to reduce the costs of brood parasitism, yet the proximate bases underlying these defenses remain poorly understood. Recent studies regarding the potential endocrine mechanisms of foreign-egg rejection have implicated corticosterone as a physiological mediator of anti-parasitic defenses. For example, corticosterone is elevated in response to non-mimetic eggs in an egg rejecter thrush, the Eurasian blackbird (*Turdus merula*) and this hormone's suppression reduces egg rejection rates in the congeneric American robin (*T. migratorius).* American robins are also among the few host species of obligate brood parasitic brown-headed cowbirds (*Molothrus ater)* that readily reject foreign eggs from their nests. We non-invasively elevated corticosterone levels in incubating female robins by dissolving it in DMSO gel which was then applied onto eggs already in the clutch. Relative to controls treated with pure DMSO gel, corticosterone-treated female robins were more likely to reject a non-mimetic, cowbird-sized foreign egg (72%) than control females (50%) when accounting for the known effect of lower clutch sizes on greater egg rejection. Future studies are needed to assess the sensory and cognitive impact(s) of corticosterone, as well as other hormones essential for parental care, in this and other hosts' defense behaviors against avian brood parasitism.

INTRODUCTION

Obligate avian brood parasites lay their eggs in the nests of other species and rely on these hosts to raise their offspring, resulting in varying reproductive costs (reviewed in Turner et al. 2021). In response, diverse host taxa of brood parasites have evolved defenses that mitigate the fitness costs associated with parasitism. Some of these defenses include vigilance behavior, aggression toward adult brood parasites, and foreign egg/nestling rejection (Soler 2017). Despite the extensive knowledge of the co-evolved counter-adaptations of hosts and brood parasites, only recently have studies started to investigate the role of physiological mechanisms that mediate host responses to parasitism and/or the presence of adult brood parasites (reviewed in Ruiz-Raya 2021). For example, those hormones that are often associated with other aggressive interactions, such as testosterone and/or progesterone, may be important for regulating attacks against brood parasites or impact host defenses in other ways (reviewed in Abolins-Abols & Hauber 2018). Examining the role of endocrine mediation of host defenses can therefore inform our understanding of how hosts respond to parasitism as well as the wide intra- and interspecific variability seen in these responses (Abolins-Abols & Hauber 2018).

Corticosterone (hereafter: CORT), is a pleiotropic glucocorticoid hormone that regulates metabolism and stress responses, among other functions (MacDougall-Shackleton et al. 2019) and has been repeatedly implicated in the responses of adult hosts to brood parasitism (Mark & Rubenstein 2013, Ruiz-Raya et al. 2018, Antonson et al. 2020). Levels of this hormone have been shown to rise in response to experimental brood parasitism with non-mimetic eggs in rejecter (Ruiz-Raya et al. 2018), but not in accepter (Scharf et al. 2021a) hosts. CORT may also affect anti-parasitic egg rejection behaviors in hosts. For example, female American robins (*Turdus migratorius;* hereafter: robins) are robust egg rejecters of both natural brown-headed cowbird (*Molothrus ater*; hereafter: cowbird) eggs and several types of model eggs, but when treated with mitotane, a glucocorticoid inhibitor, female robins rejected a deep blue, cowbird-sized egg less frequently than did control subjects (Abolins-Abols & Hauber 2020a).

Here, we further test the role of hormones in regulating egg rejection behaviors by asking if experimental elevation of CORT levels stimulates egg rejection behavior in robins. We non-invasively elevated CORT levels in female robins using a novel method of CORT incrementation pioneered by Vitousek et al. (2018) and tested their rejection responses to a deep blue, cowbird-sized model egg. This egg color is known to elicit ~50% rejection rates in robins (Hauber et al. 2020b). We predicted that female robins with elevated CORT levels would reject this same type of model egg at higher rates than control females.

METHODS

CORT Treatment

Our treatment (see validation below) consisted of powdered CORT mixed in commercially sourced dimethyl sulfoxide (DMSO; Nature's Gift^{TM}) gel, modified for robins from Vitousek et al. (2018)'s protocol for tree swallows (*Tachycineta bicolor*). For our study, we dissolved 25 mg of powdered CORT per 1 ml of DMSO gel as robins are approximately five times the mass of tree swallows; thus, we scaled up Vitousek et al. (2018)'s "high" treatment of 5 mg per 1 ml of DMSO. Our control treatment was pure DMSO gel. We then applied 300 ul of the DMSO gel collectively onto two robin eggs in each clutch, resulting in a total application of 7.5 mg CORT per clutch per application for the CORT treatment group.

Field Work and Behavioral Data Collection

We located active robin nests from April-July, 2021, in 6 plots (approximately 67,500m^2 each) at a local tree nursery in Urbana, Illinois, USA (for details, see Hauber et al. 2020b). Nests were monitored daily for clutch completion (i.e., no change in clutch size after two consecutive visits). We confirmed the incubation of nests from which the subjects were not flushed by checking the temperature of the eggs with a handheld thermometer the day following clutch completion. We then randomly applied the CORT (n=21) or pure (n=20, control) DMSO gel to the subject clutch.

Two hours after the initial treatment application, we returned to the nest to reapply the same amount of gel and added a deep blue painted, cowbird-sized model egg to the nest (Figure 2). Our aim was to elevate CORT levels before the female was experimentally parasitized, and reapplying the gel ensured that CORT levels would continue to be elevated after parasitism. We then returned to the nest 24 hours after the initial application to record the female's rejection decision (accept/reject). By this time, all gel had disappeared from the eggs, supporting our assumption that the female absorbed the gel via her brood patch. We did not monitor whether the gel treatment impacted predation, hatching success, or chick development. Nest abandonment is not a response to experimental parasitism in robins (Croston & Hauber 2014) and so nests with cold eggs (n=4) when checking for rejection and depredated nests (n=1) were excluded from our analysis. Another 4 nests were excluded from our analysis due to other reasons (i.e., gel was applied during late incubation, could not check nest the day after parasitism).

Treatment Validation

A separate set of female robins were captured using mist nets after clutch completion for treatment validation. First, gel was applied to their eggs after mist nests were set up near their nests to minimize subsequent disturbance. We then caught females following either the CORT treatment (n=7) or the control (n=7) at the two-hour mark after a single (initial) gel application. All females were bled within three minutes of capture (median = 56s, range = 41-120s) following a protocol for rapid CORT sampling in songbirds (Romero & Reed 2005). Blood samples were stored on ice in the field for up to three hours and then spun down for 10 minutes at 10,000 rpm. The plasma was then removed and stored at -20ºC for up to three months before analysis. Plasma samples (50 ul) were then sent to Creative Proteomics, Shirley, New York, USA for commercial analysis of circulating CORT levels.

Statistics

The commercial facility generated triplicate measures of CORT concentration per robin sample (median CV = 0.03, range = 0.02 - 0.05). We tested whether our DMSO gel-based treatment had a differential impact on circulating CORT levels in free-living robins by comparing control and CORT treated subjects with capture date as a predictor using a two-way ANOVA in R version 4.1.1 (R Core Team, 2021).

We then used a generalized linear mixed effect model in R with binary rejection decision (accept/reject) as the response variable (R package lme4). Treatment (CORT/control) and clutch size at the time of parasitism were included in the model as fixed predictor variables. Clutch size was previously shown to negatively impact egg rejection rates at our robin population (Abolins-Abols & Hauber 2020b). The experimental plot number indicating nest location was included as a nominal random effect in the model to account for potential spatial structuring of robin re-nesting behavior in the absence of color-banding our subjects.

Due to our *a priori* directional predictions that CORT gel increases circulating CORT concentrations and that an increase in CORT increases egg rejection rates (based on Abolins-Abols & Hauber 2020a), we analyzed both our validation and behavioral endocrine results using one-tailed statistics.

RESULTS

CORT concentrations were higher for females treated with CORT DMSO gel compared to our control of pure DMSO gel ($F = 9.36$, $df = 1$, $p < 0.01$, Figure 1), validating our treatment. CORT also increased with more advanced date of capture across our subjects ($F = 15.61$, $df = 1$, $p < 0.01$).

Females exposed to CORT DMSO gel were significantly more likely to reject a cowbird sized, deep blue egg (13 out of 18, 72%) compared to control females (7 out of 14, 50%; $\chi2 = 3.62$, $df = 1$, $p = 0.029$, Figure 2). The negative correlation between clutch sizes and egg rejection rates was marginal in our sample ($\chi2 = 2.37$, $df = 1$, $p = 0.062$).

DISCUSSION

Egg rejection is an effective defense behavior against avian brood parasitism and is increasingly known to be mediated by endocrine mechanisms (Ruiz-Raya et al. 2021). We found that female robins in the CORT treatment group were more likely to reject the deep blue model egg. This result compliments previous findings where experimentally blocking CORT resulted in decreased rejection of these model eggs (Abolins-Abols & Hauber 2020a), pointing to CORT as mechanism regulating egg rejection behavior. Our findings support this prediction: female robins in the CORT treatment group were more likely to reject the model egg when accounting for the known negative effects of increasing clutch size on egg rejection rates (Abolins-Abols & Hauber 2020b).

Egg rejection is a targeted anti-parasitic defense behavior as it requires the host to override maternal attachment to eggs in the nest (Hauber et al. 2021b). The individual variability of glucocorticoid levels and receptors could possibly explain why and/or how hosts vary in their ability to reject or respond to brood parasitism (Abolins-Abols & Hauber 2020a). As such, our results indicate that CORT mediates egg rejection in robins, but it remains to be assessed whether CORT is a relevant mediator for other egg rejection behaviors in other species as well as other anti-parasitic defenses, including nest defense and anti-parasitic mobbing.

Few studies have examined whether and how hosts initiate a stress response in the presence of brood parasites or seeing a foreign egg in the nest. Ruiz-Raya et al. (2018) found that the presence of a non-mimetic foreign egg in the nest elevates CORT levels in Eurasian blackbird (*Turdus merula*) adult females. Further, host nestlings raised alongside a parasitic nestling exhibit elevated CORT levels (Ibáñez-Àlamo et al. 2012 but see Scharf et al. 2021b). However, we note that measuring glucocorticoids is only one aspect of measuring a stress response (MacDougall Shackleton et al. 2019) and levels of other circulating hormones and biochemical markers should also be taken to further assess how hosts mount a physiological response to foreign eggs in the nest or following an encounter with an adult brood parasite.

Crucially, the non-invasive method of elevating CORT employed here, following Vitousek et al. (2018), is critical in successfully studying robins; Abolins-Abols and Hauber (2020a) reported that 40% (of n=62) females abandoned their nests after being captured and injected in the pectoral muscle, irrespective of the experimental treatment. In contrast, we only experienced 11% abandonment (4 of n=36) suggesting that invasive methods of hormone manipulations and excess handling have negative impacts on female robin's motivation to continue incubating her clutch at the time of the experiment.

Control females had noticeably higher CORT values (> 100 ng/mL) compared to other studies analyzing baseline circulating CORT levels in robins and other songbirds (e.g., Abolins and Hauber 2020a for our study population). This discrepancy could be caused by the differences between enzyme-linked immunoassay (ELISA) protocol used by Abolins-Abols and Hauber (2020a) and that used in this study (Creative Proteomics). Another possible explanation could be that the experimental protocols to set up the gel-covered eggs or capture the females resulted in elevated circulating CORT levels even in the control groups: it is possible that the females never returned to baseline levels after being disturbed to set up the mist nets. Subjects also had to incubate eggs applied with DMSO gel, which could have been a novel sensation or a possible signal of damaged eggs and, thus, initiated a CORT response. However, in this report we do not compare CORT values across studies, therefore the relative differences in CORT concentration in our commercially-measured samples are sufficient to demonstrate a statistical effect of the gel treatments on circulating hormone concentrations.

Despite recent advances (reviewed in Ruiz-Raya 2021), more research is required to determine which and how physiological mechanisms mediate host responses to brood parasitism. An integrative approach that encompasses host behavior, physiology, and endocrinology is necessary to fully understand host defense behaviors or the inability to respond to parasitism and/or adult brood parasites (Abolins-Abols & Hauber 2018). Future studies should further examine how other individual and suites of hormones may be involved in the many anti-parasitic defense mechanisms that occur in the wide array of hosts (e.g., Ruiz-Raya et al. 2021).

ACKNOWLEDGEMENTS

For funding, we are grateful to the Harley Jones Van Cleave Professorship and the Humboldt Foundation, Germany, through the University of Bielefeld (to MEH). Additional support was provided by a Graduate College Fellowship and several School of Integrative Biology awards at the University of Illinois to (AMT). All research was conducted on private property (Wandell's Tree Farm) and approved by federal, state, and institutional animal research permits. For help and discussions, we are grateful to Maren Vitousek, Sarah Winnicki, Wendy Schelsky, and Jeff Hoover.

FIGURES

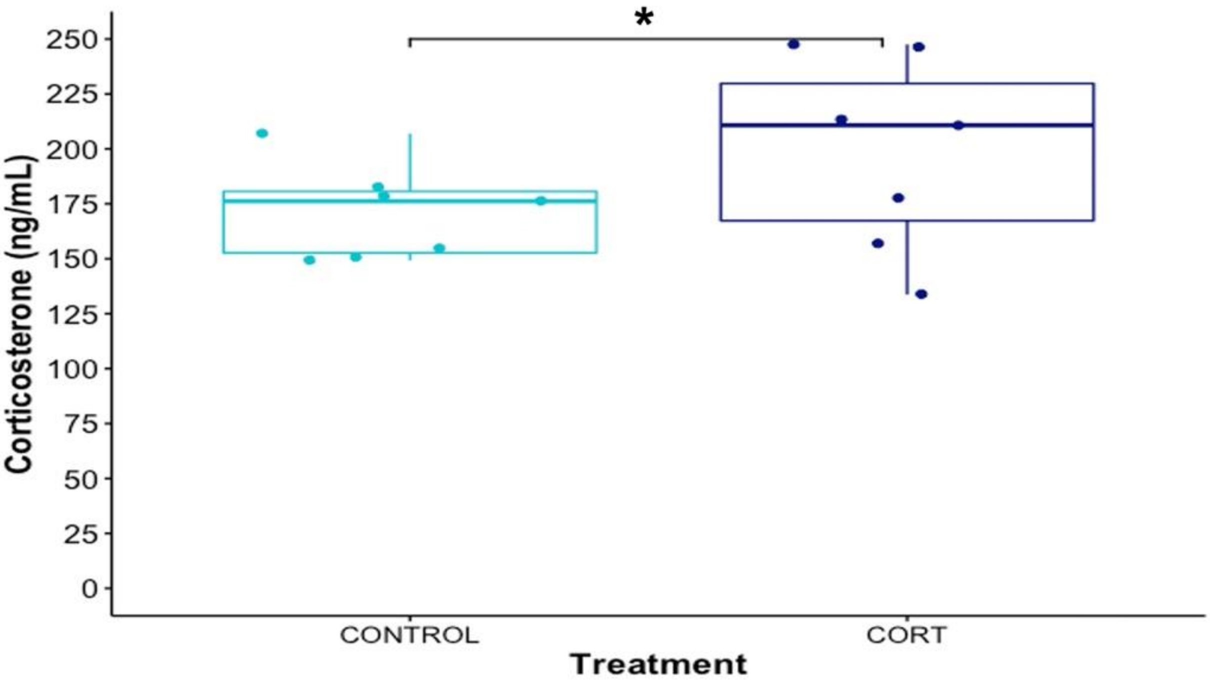

Figure 5.1. Corticosterone (ng/mL) values for control (n=7) and cort treated (n=7) female American robins. The Asterix represents one-tailed statistical significance between the two treatments, when controlling for date of experimentation, and the box plots indicate 10th, 25th, 50th, 75th, and 90th percentiles in addition to dots indicating all data points.

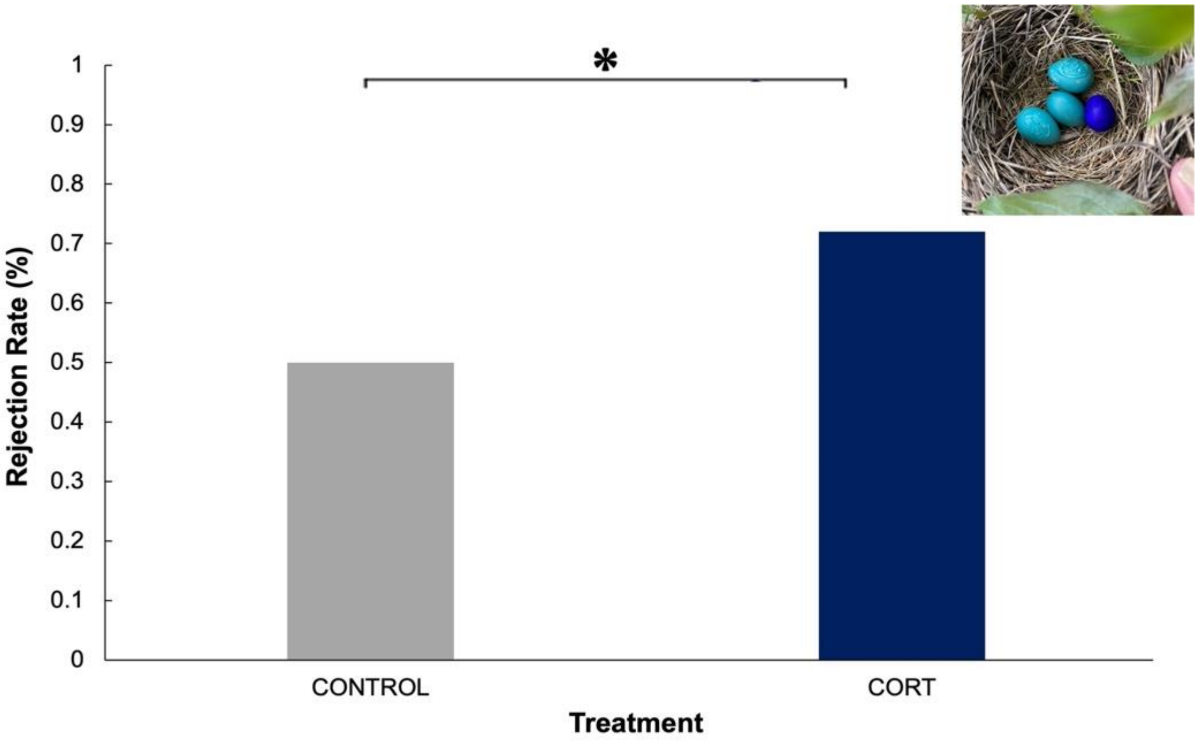

Figure 5.2. Rejection rates by American robins between control (n=14) and CORT (n=18) treatments. Asterix represents one-tailed statistical significance between the two treatments, when controlling for clutch size. Photo in top right shows gel treatment applied to two robin eggs and a deep-blue, cowbird-sized foreign egg.

REFERENCES

Abernathy VE and Peer BD (2015) Mechanisms of egg recognition in brown-headed cowbird hosts: the role of ultraviolet reflectance. Animal Behaviour, 109: 73-79.

Abolins-Abols M, Hanley D, Moskát C, Grim T, and Hauber ME (2019) Anti-parasitic egg rejection by great reed warblers (*Acrocephalus arundinaceus)* tracks differences along an eggshell color gradient. Behavioural Processes, 166: 103902.

Abolins-Abols M and Hauber ME (2018) Host defenses against avian brood parasitism: an endocrine perspective. Proceedings of the Royal Society B, 285: 20180980.

Abolins-Abols M and Hauber ME (2020a) Endocrine regulation of egg rejection in an avian brood parasite host. Biology Letters, 16: 20200225.

Abolins-Abols M and Hauber ME (2020b) Proximate predictors of variation in egg rejection behavior by hosts of avian brood parasites. Journal of Comparative Psychology, 134: 412-422.

Aidala Z, Croston R, Schwartz J, Tong L, and Hauber ME (2015) The role of egg-nest contrast in the rejection of brood parasitic eggs. Journal of Experimental Biology, 218: 1126–1136.

Aidala Z, Huynen L, Brennan PLR, Musser J, Fidler A, Chong N, Machovsky Capuska GE, Anderson MG, Talaba A, Lambert D, and Hauber ME (2012) Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 198: 495-510.

Alvarez F, Arias De Reyna L, and Segura M (1976) Experimental brood parasitism of the magpie (*Pica pica).*Animal Behaviour, 24: 907-916.

Anderson MG, Gill BJ, Briskie JV, Brunton DH, and Hauber ME (2013) Latitudinal differences in the breeding phenology of Grey Warblers covary with the prevalence of parasitism by Shining Bronze-Cuckoos. Emu, 113: 187–191.

Antonov A, Stokke BG, Moksnes A, and Røskaft E (2007) Getting rid of the cuckoo *Cuculus canorus* egg: why do hosts delay rejection? Behavioral Ecology, 19: 100–107.

Antonson ND, Hauber ME, Mommer BC, Hoover JP, Schelsky WM (2020) Physiological responses of host parents to rearing an avian brood parasite: an experimental study. Hormones and Behavior, 125: 104812.

Avilés JM and Parejo D (2011) Host personalities and the evolution of behavioural adaptations in brood parasitic-host systems. Animal Behaviour, 82: 613-618.

Avilés JM, Soler JJ, and Pérez-Contreras T (2006) Dark nests and egg colour in birds: a possible functional role of ultraviolet reflectance in egg detectability. Proceedings of the Royal Society B: Biological Sciences, 273: 2821-2829.

Bán M, Moskát C, Barta Z, and Hauber ME (2013) Simultaneous viewing of own and parasitic eggs is not required for egg rejection by a cuckoo host. Behavioral Ecology, 24: 1014–1021.

Bell AM, Hankison SJ, and Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Animal Behaviour. 77: 771-783.

Bazin RC (1991) Defenses against avian brood parasitism in the Eastern Kingbird. M.Sc. Thesis, University of Manitoba, Winnipeg, MB.

Blair RH and Tucker BW (1941) Nest sanitation. British Birds, 34: 206-215.

Briskie JV, Sealy SG, and Hobson KA (1992) Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. Evolution, 46: 334–340.

Butler MW, Stine ME, and Ki KC (2020) Eastern bluebirds (*Sialia sialis)* use color patterning, but not the colors themselves as a cue to eject interspecific parasitic eggs. Condor, 137: 1-9.

Campobello D and Sealy SG (2018) Evolutionary significance of antiparasite, antipredator and learning phenotypes of avian nest defense. Scientific Reports, 8: 10569.

Carmody LC, Cruz A, and Chace JF (2016) Brood parasitism defense behaviors along an altitudinal gradient in the American robin (*Turdus migratorius*). The Open Ornithology Journal, 9: 39–49.

Canniff L, Dainson M, López AV, Hauber ME, Grim T, Samaš P, & Hanley D (2018) Probing the limits of egg recognition using egg rejection experiments along phenotypic gradients. Journal of Visualized Experiments, 138: e57512.

Cassey P, Honza M, Grim T, and Hauber ME (2008) The modelling of avian visual perception predicts behavioural rejection responses to foreign egg colours. Biology Letters, 4: 515–517.

Croston R and Hauber ME (2014a) High repeatability of egg rejection in response to experimental brood parasitism in the American robin (*Turdus migratorius*). Behaviour, 151: 703.

Croston R and Hauber ME (2014b) Spectral tuning and perceptual differences do not explain the rejection of brood parasitic eggs by American robins (*Turdus migratorius*). Behavioral Ecology and Sociobiology, 68: 351–362.

Croston R and Hauber ME (2015a) 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*). Ethology Ecology & Evolution, 27: 42–55.

Croston R and Hauber ME (2015b) Experimental shifts in intraclutch egg color variation do not affect egg rejection in a host of a non-egg-mimetic avian brood parasite. PloS ONE, 10: 1–14.

Dainson M, Hauber ME, López AV, Grim T, and Hanley D (2017) Does contrast between eggshell ground and spot coloration affect egg rejection? Science of Nature, 104: 54.

Davies NB and de L Brooke M (1988) Cuckoos versus reed warblers: Adaptations and Counteradaptations. Animal Behaviour, 36: 262-284.

Davies NB and de L Brooke M (1989) An experimental study of co-evolution between the cuckoo, *cuculus canorus*, and its hosts. I. host egg discrimination. Journal of Animal Ecology, 58: 207–224.

Dinets V, Samaš P, Croston R, Grim T, and Hauber ME (2015) Predicting the responses of native birds to transoceanic invasions by avian brood parasites. Journal of Field Ornithology, 86: 244–251.

Endler JA and Mielke PW (2005) Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society, 86: 405–431.

Feeney WE and Langmore NE (2013) Social learning of a brood parasite by its host. Biology Letters, 9: 20130443.

Feeney WE, Welbergen JA, and Langmore NE (2012) The frontline of avian brood parasite-host coevolution. Animal Behaviour, 84: 3-12.

Franklin WE and Lima SL (2001) Laterality in avian vigilance: do sparrows have a favourite eye? Animal Behaviour, 62: 879–885.

Friedmann H (1929) The cowbirds: a study in the biology of social parasitism. Springfield, IL: Charles C. Thomas Publisher.

Geltsch N, Bán M, Hauber ME, and Moskát C (2016) Optimal timing of egg laying by Common Cuckoos *Cuculus canorus*: when should parasitic eggs be laid to precede the host eggs in hatching? Bird Study, 63: 46-51.

Golüke S, Dörrenberg S, Krausse ET, Caspers BA (2016) Female zebra finches smell their own eggs. *PLoS* ONE, 11: e0155513.

Grim T, Samaš P, and Hauber ME (2014) The repeatability of avian egg ejection behaviors across different temporal scales, breeding stages, female ages and experiences. Behavioral Ecology and Sociobiology. 68: 749-759.

Guigueno MF and Sealy SG (2009) Nest sanitation plays a role in egg burial by yellow warblers. Ethology, 115: 247–256.

Guigueno MF and Sealy SG (2012) Nest sanitation in passerine birds: implications for egg rejection in hosts of brood parasites. Journal of Ornithology, 153: 35-52.

Haas CA and Haas KH (1998) Brood parasitism by brown-headed cowbirds on brown thrashers: frequency and rates of rejection. The Condor, 100: 535–540.

Hanley D, Grim T, Igic B, Samaš P, López AV, Shawkey MD and Hauber ME (2017) Egg discrimination along a gradient of natural variation in eggshell coloration. Proceedings of the Royal Society B: Biological Sciences, 284: 20162592.

Hanley D, López AV, Fiorini VD, Reboreda JC, Grim T and Hauber ME (2019) Variation in multicomponent recognition cues alters egg rejection decisions: a test of the optimal acceptance threshold hypothesis. Philosophical Transactions Royal Society, 374: 20180195.

Hanley D, Samaš P, Heryan J, Hauber ME, and Grim T (2015) Now you see it, now you don't: flushing hosts prior to experimentation can predict their responses to brood parasitism.—Scientific Report, 5: 9060.

Hauber ME (2003a) Egg-capping is a cost paid by hosts of interspecific brood parasites. The Auk, 120: 860-865.

Hauber ME (2003b) Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. Behavioral Ecology, 14: 227–235.

Hauber ME (2020) Natural and artificial scents do not increase egg rejection rates of model brood parasitic eggs by American robins (*Turdus migratorius).* Acta Zoologica Academiae Scientiarum Hungaricae, 66: 309–317.

Hauber ME, Abolins-Abols M, Kim CR and Paitz RT (2020a) Inter-individual variation in anti-parasitic egg rejection behavior: A test of the maternal investment hypothesis. Integrative Organismal Biology, 2: 1-10.

Hauber ME, Hoover JP, Rhodes OR, Ducay RL, and Hanley D (2021a) The blunt pole is not a source of more salient recognition cues than the sharp pole for the rejection of model eggs by American robins (*Turdus migratorius).* Journal of Vertebrate Biology, 70: 20111.

Hauber ME, Moskát C and Bán M (2006) Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. Biology Letters, 2: 177–180.

Hauber ME, Tong L, Bán M, Croston R, Grim T, Waterhouse GIN, Shawkey MD, Barron AB and Moskát C (2015) The value of artificial stimuli in behavioral research: making the case for egg rejection studies in avian brood parasitism. Ethology, 121: 521–528.

Hauber ME, Dainson M, Luro A, Louder AA and Hanley D (2019) When are egg-rejection cues perceived? A test using thermochromic eggs in an avian brood parasite host. Animal Cognition, 22: 1141–1148.

Hauber ME, Kim CR, Goethe C and Hanley D (2020b) Self-referent phenotype matching is a poor predictor of egg rejection by American Robins. Journal of Field Ornithology, 91: 254–262.

Hauber ME, Winnicki SK, Hoover JP, Hanley D and Hays IR (2021b) The limits of egg recognition: Testing the acceptance thresholds of American robins in response to decreasingly egg-shaped objects in nest. Royal Society Open Science 8: 201615.

Hebets EA, Barron AB, Balakrishnan CN, Hauber ME, Mason PH and Hoke KL (2016) A systems approach to animal communication. Proceedings of the Royal Society B: Biological Sciences, 283: 1–10.

Hoover JP and Robinson SK (2007) Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. PNAS, 104: 4479-4483.

Hosoi SA and Rothstein SI (2000) Nest desertion and cowbird parasitism: Evidence for evolved responses and evolutionary lag. Animal Behaviour, 59: 823–840.

Ibáñez-Álamo JD, De Neve L, Roldán M, Rodríguez J, Trouvé C, Chastel O, Soler M (2020) Corticosterone levels in host and parasite nestlings: is brood parasitism a hormonal stressor? Hormones and Behavior, 61: 590-597.

Ibáñez-Álamo JD, Ruiz-Raya F, Roncalli G, and Soler M (2014) Is nest predation an important selective pressure determining fecal sac removal? The effect of olfactory cues. Journal of Ornithology, 155: 491-496.

Igic B, Nunez V, Voss HU, Croston R, Aidala Z, López AV, Tatenhove AV, Holford ME, Shawkey MD and Hauber ME (2015) Using 3D printed eggs to examine the egg-rejection behaviour of wild birds. PeerJ , 3: e965.

Jamie GA, Van Belleghem SM, Hogan BG, Hamama S, Moya C, Troscianko J, Stoddard MC, Kilner RM and Spottiswoode CN (2020) Multimodal mimicry of hosts in a radiation of parasitic finches. Evolution, 74: 2526-2538.

Jammalamadaka SR and SenGupta A (2001) Circular statistics.—In: Topics in Circular Statistics. World Scientific Publishing, Singapore, p. 291-293.

Kemal RE and Rothstein SI (1988) Mechanisms of avian egg recognition: Adaptive responses to eggs with broken shells. Animal Behaviour, 36: 175–183.

Lang AK, Bollinger EK and Peer BD (2014) Effect of parasite-to-host egg ratio on egg rejection by a Brown-headed Cowbird host. Auk, 131: 694–701.

Lang JD, Straight CA, and Gowaty A (2002) Observations of fecal sac disposal by eastern Bluebirds. The Condor, 104: 205-207.

Lee JW, Kim DW and Yoo JC (2005) Egg rejection by both male and female vinous-throated parrotbills *Paradoxornis webbianus*. Integrative Biosciences, 9: 211-213.

Lotem A, Hiroshi N, and Zahavi A (1992) Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. Behavioral Ecology. 3: 128-132.

Lotem A, Nakamura H and Zahavi A (1995) Constraints on egg discrimination and cuckoo-host co-evolution. Animal Behaviour, 48: 1185-1209.

Lowther PE (2020) Brown-headed cowbird (*Molothrus ater)*. In Birds of the World (AF Poole and FB Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.

Luro AB and Hauber ME (2017) A test of the nest sanitation hypothesis for the evolution of foreign egg rejection in an avian brood parasite rejecter host species. The Science of Nature, 104: 14.

Luro AB and Hauber ME (2020) The ecological context and fitness impact of categorical perception: a comment on Green et al. Behavioral Ecology, 31: 869-870.

Luro AB, Igic B, Croston R, López AV, Shawkey MD and Hauber ME (2018) Which egg features predict egg rejection responses in American robins? Replicating Rothstein's (1982) study. Ecology and Evolution, 8: 1673–1679.

Luro AB, Fernández-Juricic E, Baumhardt P, and Hauber ME (2020) Visual acuity and egg spatial chromatic contrast predict egg rejection behavior of American robins. Journal of Experimental Biology, 223: jeb2296909.

Macdougall-Shackelton SA, Bonier F, Romero LM, and Moore IT (2019) Glucocorticoids and "stress" are not synonymous. Integrative Organismal Biology, 1: 1-8.

Manna T, Moskát C, and Hauber ME (2017) Cognitive decision rules for egg rejection. In M. Soler (ed.), Avian Brood Parasitism (pp. 438-448). New York: Springer Nature.

Manna TJ, Hanley D, Honza M, Capek M, Rutila J, Samaš P, Abolins-Abols M, and Hauber ME (2020) Fitting different visual models to behavioral patterns of parasitic egg rejection along a natural egg color gradient in a cavity-nesting host species. Vision Research, 167: 54-59.

Mark MM and Rubenstein DR (2013) Physiological costs and carry over effects of avian interspecific brood parasitism influence reproductive tradeoffs. Hormones and Behavior, 63: 717-722.

Martínez JG, Molina-Morales M, Precioso M, and Avilés JM (2020) Age-related brood parasitism and egg rejection in magpie hosts. American Naturalist, 195: 876-885.

Mason P & Rothstein SI (1986) Coevolution and avian brood parasitism: cowbird eggs show evolutionary response to host discrimination. Evolution, 40: 1207-1214.

Medina I and Langmore NE (2019) Nest illumination and the evolution of egg rejection in hosts of brood parasites. Auk, 136: 1-6.

Molina-Morales M, Martínez JG, Martín-Gálvez D, Dawson DA, Burke T and Avilés JM (2014) Cuckoo hosts shift from accepting to rejecting parasitic eggs across their lifetime. Evolution, 68: 3020–3029.

Møller AP and Si X (2021) Personality of hosts and their brood parasites. Current Zoology. 67: 625-630.

Moskát C, Bán M, and Hauber ME (2014a) Naïve hosts of avian brood parasite eggs, whereas older hosts fine-tune foreign egg discrimination during laying. *Frontiers in Zoology*, 11, 45.

Moskát C, Székely T, Kisbenedek T, Karcza Z, and Bártol I (2003) The importance of nest cleaning in egg rejection behaviour of great reed warblers *Acrocephalus arundinaceus*. Journal of Avian Biology, 34: 16-19.

Moskát C, Zölei A, Bán M, Elek Z, Tong L, Geltsch N, and Hauber ME (2014b) How to spot a stranger’s egg? A mimicry-specific discordancy effect in the recognition of parasitic eggs. Ethology, 120: 616–626.

Ouyang JQ, Quetting M, and Hau M (2012) Corticosterone and brood abandonment in a passerine bird. Animal Behaviour, 84: 261-268.

Peer BD, Robinson S, and Herkert J (2000) Egg rejection by cowbird hosts in grasslands. Auk, 117: 892–901.

Peer BD, Rothstein SI, and Mccleery RA (2010) Intraclutch variation in egg appearance constrains rejection of brown-headed cowbird (*Molothrus ater*) eggs in common grackles (*Quiscalus quiscula*). Auk, 127: 759–764.

Peer BD and Sealy SG (2004a) Correlates of egg rejection in hosts of the brown-headed cowbird. The Condor, 106: 580–599.

Peer BD and Sealy SG (2004b) Fate of grackle (*Quiscalus spp.*) defenses in the absence of brood parasitism: Implications for long-term parasite-host coevolution. The Auk, 121: 1172-1186.

Petit KE, Petit LJ, and Petit DR (1989) Fecal sac removal: do the pattern and distance of dispersal affect the chance of the nest predation? The Condor, 91: 479-482.

Pribil S and Picman J (1997) Parasitism of house wren nests by brown-headed cowbirds: why is it so rare? Canadian Journal of Zoology, 75: 302–307.

Procházka P, Konvičkova-Patzenhauerová H, Požgayová M, Trnka A, Jelínek V, and Honza M (2014) Host genotype and age have no effect on rejection of parasitic eggs. Naturwissenschaften. 101: 417-426.

Polačiková L and Grim T (2010) Blunt egg pole holds cues for alien egg discrimination: experimental evidence. Journal of Avian Biology, 41: 111-116.

Quan RC, Huan L, Wang B, and Goodale E (2022) The relationship between defecation and feeding in nestling birds: observational and experimental evidence. Frontiers in Zoology, 12: 21.

R Foundation for Statistical Computing (2021) R: A language and environment for statistical computing. R version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria.

Rasmussen JL, Sealy SG, and Underwood TJ (2009) Video recording reveals the method of ejection of brown-headed cowbird eggs and no cost in American robins and gray catbirds. The Condor, 111: 570–574.

Reeve HK (1989) The evolution of conspecific acceptance thresholds. American Naturalist, 133: 407–435.

Rohwer S and Spaw CD (1988) Evolutionary lag versus bill-size constraints: A comparative study of the acceptance of cowbird eggs by old hosts. Evolutionary Ecology, 2: 27-36.

Romero LM and Reed JM (2005) Collecting baseline corticosterone samples in the field: is under 3 minutes good enough? Comparative Biochemistry and Physiology, 140: 73-79.

Rothstein SI (1975a) An experimental and teleonomic investigation of avian brood parasitism. The Condor, 77: 250–271.

Rothstein SI (1975b) Mechanisms of avian egg-recognition: Do birds know their own eggs? Animal Behaviour, 23: 268–278.

Rothstein SI (1982) Mechanisms of avian egg recognition: Which egg parameters elicit responses by rejecter species? Behavioral Ecology and Sociobiology, 11: 229–239.

Rowe KMC and Weatherhead PJ (2009) A third incubation tactic: Delayed incubation by American Robins (*Turdus migratorius).* The Auk, 126: 141-146.

Rubio E, Sanllorente O, Tieleman BI, and Ibáñez-Álamo JD (2018) Fecal sacs do not increase nest predation in a ground nester. Journal of Ornithology, 159: 985-990.

Ruiz-Raya F (2021) Ecophysiology of egg rejection in hosts of avian brood parasites: new insights and perspectives. Current Zoology, 67: 631-638.

Ruiz-Raya F, Ibáñez-Álamo J, Parenteau C, Chastel O, and Soler M (2021) Prolactin mediates behavioural rejection responses to avian brood parasitism. Journal of Experimental Biology, 224: jeb240101.

Ruiz-Raya F and Soler M (2020) Signal detection and optimal acceptance thresholds in avian brood parasite–host systems: Implications for egg rejection. Philosophical Transactions of the Royal Society B: Biological Sciences, 375: 20190477.

Ruiz-Raya F, Soler M, Abaurrea T, Chastel O, Roncalli G, and Diego J (2018) Hormonal responses to non-mimetic eggs: is brood parasitism a physiological stressor during incubation? Behavioral Ecology and Sociobiology, 72: 153.

Ruiz-Raya F, Soler M, Roncalli G and Ibáñez-Álamo JD (2019) Egg-recognition abilities in non-incubating males: implications for the evolution of anti-parasitic host defenses. Behavioral Ecology and Sociobiology, 73: 17.

Ruiz-Raya F, Soler M, Sánchez-Pérez L Ll, and Ibáñez-Álamo JD (2015) Could a factor that does not affect egg recognition influence the decision of rejection? PLoS ONE, 10: e0135624.

Rutledge S, Carr DE, Hauber ME and Hanley D (2021) Best of a bad job or masters of illusion: do nest light conditions make the eggs of brood parasitic brown-headed cowbirds (*Molothrus ater*) more similar to the eggs of its hosts? Ethology, 127: 117-124.

Samaš P, Hauber ME, and Honza M (2021) A meta-analysis of avian eggshell characteristics cueing egg-rejection defenses against brood parasitism. Frontiers in Ecology and Evolution, 9: 703208.

Sato NJ, Tokue K, Noske RA, Mikami OK and Ueda K (2010) Evicting cuckoo nestlings from the nest: A new anti-parasitism behaviour. Biology Letters, 6: 67–69.

Scharf HM, Abolins-Abols M, Stenstrom KH, Tolman DT, Schelsky WM, and Hauber ME (2021a) Exposure to a mimetic or non-mimetic model avian brood parasite egg does not produce differential glucocorticoid responses in an egg-accepter host species. General and Comparative Endocrinology, 304: 113723.

Scharf HM, Hauber ME, Mommer BC, Hoover JP, and Schelsky WM (2021b) The effect of avian brood parasitism on physiological responses of host nestlings. Oecologia, 195: 861-872.

Scharf HM, Stenstrom K, Dainson M, Benson TJ, Fernández-Juricic E, and Hauber ME (2019) Mimicry-dependent lateralization in the visual inspection of foreign eggs by American robins. Biology Letters, 15: 20190351.

Scharf HM, Suarez AV, Reeve HK, and Hauber ME (2020) The evolution of conspecific acceptance threshold models. Philosophical Transactions of the Royal Society of London B, 375: 20190475.

Sealy SG (1996) Evolution of host defenses against avian brood parasitism: Implications of puncture-ejection by a small passerine. The Auk, 113: 346-355.

Sealy SG and Neudorf DL (1995) Male northern orioles eject cowbird eggs: implications for the evolution of rejection behavior. Condor, 97: 369-275.

Shaw RC, Feeney WE, & Hauber ME (2014) Nest destruction elicits indiscriminate con- vs. heterospecific brood parasitism in a captive bird. Ecology & Evolution, 4: 4500-4504.

Soler M (2017) Avian Brood Parasitism: Behavior, Ecology, Evolution, and Coevolution. Switzerland: Springer International Publishing.

Soler M, Soler JJ, Martinez JG, and Møller AP (1995) Magpie host manipulation by great spotted cuckoos: Evidence for an avian mafia? Evolution, 49: 770-775.

Soler JJ, Pérez-Contreras T, Neve LD, Macías-Sánchez E, Møller AP, and Soler M (2014) Recognizing odd smells and ejection of brood parasitic eggs. An experimental test in magpies of a novel defensive trait against brood parasitism. Journal of Evolutionary Biology, 27: 1265–1270.

Stevens M, Troscianko J, and Spottiswoode CN (2013) Repeated targeting of the same hosts by a brood parasite compromises host egg rejection. Nature Communications, 4: 2475.

Stoffel MA, Nakagawa S, and Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods in Ecology and Evolution. 8: 1639-1644.

Strausberger BM (1998) Temperature, egg mass, and incubation time: A comparison of brown-headed cowbirds and red-winged blackbirds. The Auk, 115: 843-850.

Strausberger BM and Hauber ME (2017) Encounters with adult brood parasitic cowbirds at nests, not costly parasitic eggs, elicit nest desertion by Chipping Sparrows (*Spizella passerina*).Wilson Journal of Ornithology, 129: 554-559.

Tinbergen N, Broekhuysen J, Feekes F, Houghton JCW, Kruuk H, and Szulc E (1962) Egg shell removal by the Black-headed Gull *Larus ridibundus* L.; A behaviour component of camouflage. Behaviour, 19: 74-177.

Thorogood R and Davies NB (2012) Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. Science, 337: 578–580.

Tosi-Germán RA, Tassino B, and Reboreda JC (2020) Female and male rufous horneros eject shiny cowbird eggs using a mental template of the size of their own eggs. Behavioural Processes, 178: 104152.

Turner AM, Di Giovanni AJ, Antonson ND, Scharf HM, Abolins-Abols M, and Hauber ME (2022) Non-invasive elevation of circulating corticosterone increases the rejection of foreign eggs in female American robins (*Turdus migratorius)*. Hormones and Behavior, 146: 105278.

Turner AM and Hauber ME (2021) The American robin (*Turdus migratorius):* A focal species for anti-parasitic egg rejection studies among hosts of the brown-headed cowbird (*Molothrus ater).* Ethology, 127: 490-503.

Turner AM, Robinson SK, Hauber ME (2021) Avian Brood Parasitism. Encyclopedia of Biodiversity, 3rd Edition (Cardoso da Silva JM, editor), Elsevier, London.

Turner AM, Sluis VN, Williams EJ, Benson TJ, Ward MP, and Hauber ME (2023) Examining causes and characteristics of ejection behaviour in the American robin (*Turdus migratorius)*. Behaviour. 160: 617-633.

Underwood TJ and Sealy SG (2006) Influence of shape on egg discrimination in American robins and gray catbirds. Ethology, 112: 164–173.

Vanderhoff N, Pyle P, Patten MA, Sallabanks R, and James FC (2020) American Robin (*Turdus migratorius).* In Birds of the World (PG Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY.

Vitousek MN, Taff CC, Ardia DR, Stedman JM, Zimmer C, Salzman TC, and Winkler DW (2018) The lingering impact of stress: brief acute glucocorticoid exposure has sustained, dose dependent effects on reproduction. Proceedings of the Royal Society B, 285: 20180722.

Ward D, Lindholm AK, and Smith JNM (1996) Multiple parasitism of the red-winged blackbird: Experimental evidence of evolutionary lag in a common host of the brown-headed cowbird. Auk, 113: 408-413.

Weitzel NH (2003) Western bluebird (*Sialia mexicana)* fecal sac dispersal at Kellogg, Oregon. Western North American Naturalist, 63: 268-270.

Weitzel NH (2005) White-breasted nuthatch (*Sitta carolinensis)* fecal sac disposal in Northwestern Nevada. Western North American Naturalist, 65: 229-232.

Welbergen JA and Davies NB (2009) Strategic variation in mobbing as a front line of defense against brood parasitism. Current Biology, 19: 235–240.

Winfree R (1999) Cuckoos, cowbirds and the persistence of brood parasitism. Trends in Ecology and Evolution, 14: 338–343.

Winnicki SK, Hauber ME, Benson TJ, and Abolins-Abols M (2022) Ground nesting by arboreal American robins (*Turdus migratorius). Ecology and Evolution*, 12: e8489.

Xie Y (2014) knitr: A comprehensive tool for reproducible research in R.—In: Implementing reproducible computational research (Stodden V, Leisch F, Peng RD, eds.). Chapman and Hall/CRC, p. 29

Xu K, Servedio MR, Winnicki SK, Moskát C, Hoover JP, Turner AM, Hauber ME (2023) Host learning selects for the coevolution of greater egg-mimicry and narrower anti-parasitic egg-rejection thresholds. Evolution Letters. [online]

Yang Q, Chen X, Zhang Z, Han J, Wu N, and Yang C (2022) Egg rejection and nest sanitation in an island population of barn swallows (*Hirundo rustica):* Probability, response latency, and sex effects. Animals, 12: 3027.

Zalasiewicz J and Williams M (2012) The Goldilocks planet: The 4 billion year story of earth's climate. Oxford University Press, NY, USA.

Zhang J, Santema P, Li J, Yang L, Deng W, and Kempenaers B (2021) Host personality predicts cuckoo egg rejection in Daurian redstarts *Phoenicurus auroreus.* Proceedings of the Royal Society B, 288: 20210228.