Cognitive Models of Defense Behaviors in Hosts of Brood Parasites

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by

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Abstract

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Social parasites exploit the behavioral repertoire of their hosts for their own benefit, thereby reducing host reproductive success. Whether and how hosts respond to prevent, reduce, or eliminate the costs of parasitism requires the characterization of host cognitive algorithms in response to parasites. In this dissertation, I review the suite of the defense behaviors and decision rules of hosts targeted by avian and insect brood parasites, and present new experimental data on the detection of parasitism through the visual system of focal host species. In Chapter 1, I review extensive data already accumulated to isolate the cognitive mechanisms used by avian hosts to assess, identify, and reject foreign eggs in the nest. The two most commonly evoked candidate heuristics are the discordancy mechanism, wherein the host rejects the egg most dissimilar in appearance to the other eggs in the clutch, and the template-based mechanism, wherein the host compares an egg to an internal template of its own eggs’ appearance. When experimenters directly pitted these competing mechanisms against one another, they found dominant support of the template-based process for egg discrimination. More recent and detailed analyses, however, to tease these mechanisms apart suggested that these alternatives are not mutually exclusive, and may be simultaneously activated to work in tandem to effect egg rejection decisions. Furthermore,
Chapter 1 overviews a growing body of work demonstrating, at the individual level, the extent of plasticity that exists in setting the phenotypic threshold for own-foreign discrimination by hosts.

To examine egg rejection behaviors experimentally, the potential host species investigated must meet certain requirements. These include that they be rejecters of foreign eggs to some level of predictability and consistency in the first place. The globally ubiquitous house sparrow *Passer domesticus* is known to engage in conspecific brood parasitism, and its invasive proliferation across many continents and biomes represents a potentially attractive system to study anti brood parasite behaviors across ecological contexts and scales. Chapter 2, therefore, assesses the generalizability of previous studies demonstrating egg rejection patterns in house sparrows in Spain and South Africa, especially following the publication of more recent data from China suggesting that this species is not an egg rejecter. Here we robustly examined house sparrow responses to experimental parasitism in the distinct regions of North America, Israel, and New Zealand, and found negligible rejection rates in all three, suggesting that the house sparrow is not a suitable global model for antiparasitic egg rejection behaviors.

In Chapter 3, the cognitive mechanisms of rejection responses characterized in chapter 1 were experimentally tested by analyzing diverse published and unpublished datasets from the great reed warbler *Acrocephalus arundinaceus*, a well-studied host species of a mimetic
race of the obligate brood parasitic common cuckoo *Cuculus canorus*. Specifically, the simultaneous activation of the discordancy and template-based decision rules suggested in chapter 1 was considered to test whether multiple methods were employed at the same time in a way that they may have interfered with one another to reduce rejection accuracy. Host individuals were experimentally parasitized with painted eggs of varying colors, quantities, and uniformities. Hosts were found to be more permissive of foreign eggs, and thus more error prone, when both the proportion of foreign eggs the nest increased and the eggs in the nest became more perceptually distinct from one another. This indicates that host defenses could be compromised by causing recognition mechanisms to yield differing rejection targets, and that multiple parasitism (or repeated targeting: more than one parasitic egg laid in the host nest) can mediate this beneficial outcome for the parasite itself.

No matter which cognitive egg rejection mechanism(s) is(are) employed, most studies agreed that the primary visual cue used by hosts to distinguish foreign eggs is the overall degree of distance in color between the egg being assessed and the host’s own egg(s). Until recently, rejection decisions were attributed to the absolute (regardless of direction) perceptual distance between own vs. foreign eggs. Chapter 4 is a new original but also parallel study to Hanley et al. 2017 and 2019’s discoveries that directional difference on a continuous color gradient of avian eggshell colors may be the relevant salient recognition cue. In particular, Hanley et al. found that their hosts preferentially rejected eggs browner than their own, but not eggs more blue/green than their own, suggesting a single threshold
of rejection only on one side of the natural avian color gradient, rather than multiple symmetrical thresholds of absolute distance. We examined this phenomenon focusing on the European redstart *Phoenicurus phoenicurus*, which in contrast to the house sparrow observed in Chapter 2, consistently demonstrated rejection of non-mimetic eggs and acceptance of mimetic eggs in prior studies, thereby providing an attractive subject for variable egg rejection rates to investigate the limits of color-based rejection threshold(s). In addition, this study assessed Hanley et al.’s hypothesis in the context of a host species parasitized by a mimetic parasite race, which none of their prior studies included. Specifically, we experimented with redstarts in Finland, where they were simultaneously under parasitic pressure from the common cuckoo, and in the Czech Republic, where no parasitic pressure was present. Using 3D printed eggs painted along a continuous color gradient of natural brown to blue/green avian eggshell background colors, we experimentally parasitized redstart nests and recorded their rejection behaviors. In support of the single threshold model, we found the redstarts, regardless of locality, preferentially rejected noticeably browner eggs but not noticeably more blue/green eggs.

Finally, in Chapter 5, I shift the lens to the insect kingdom to examine highly analogous host vs. brood parasite systems to what we have seen in birds. Though the exact antiparasitic sensory modalities and recognition mechanisms differ, the evolutionary arms race of mimicry and recognition as parasites attempt to exploit unrelated individuals for offspring care is remarkably similar, and the relative advantages of examining host-parasite
interactions from this new perspective are carefully enumerated. Relative to avian brood parasitism, the study of social parasitism in insects is still patchy, even when there are strong analogies (such as parasitic larvae manipulating caretakers to receive disproportionate attention, just as many avian brood parasite chicks manipulate foster parents to receive biasedly greater share of the provisioning), yet the sensory mechanisms of the larval manipulation remain largely unknown. Making use of these multiple perspectives on host-parasite dynamics across taxa can inspire more cohesive research across taxonomic boundaries. Such work then also inspires both conceptual advancement and applied analyses, for example, in the context of an impending conservation crisis as the collapse of honeybee colonies in Africa accelerates due to the recent surge of virulence of its brood parasitic congener.

All but Chapter 4 of this dissertation represent peer-reviewed and published articles that have already appeared in print and online as Manna et al. Chapter 4 in turn, will be the basis of a new manuscript with the same first author and institutional affiliation, yet again.
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Chapter 1: Cognitive Decision Rules for Egg Rejection

Cognitive Decision Rules for Egg Rejection

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Abstract

Egg rejection is the best studied behavioral adaptation by hosts to avian brood parasitism. Investigations of the mechanism(s) by which a host accomplishes the task of perceiving and deciding to reject a foreign egg have been a hotbed of debate and discovery for decades. The two most often tested cognitive explanations for this behavior are: 1) The host rejects the egg most dissimilar from the other eggs in the nest (discordancy mechanism) and 2) The host compares each egg to an internal template of the appearance of its own eggs (template recognition mechanism). While many years of published work have purported sole support for the template recognition hypothesis (for instance, hosts can experimentally reject foreign eggs which do not represent a quantitative minority in the clutch), in recent years an increasingly prevalent argument that the two mechanisms are working in tandem has come to light. Furthermore, there is also a steadily building body of work indicating that hosts have plastic discrimination thresholds, such that the extent to which parasitic eggs must be different from a host’s own egg before rejection occurs appears to be both socio-ecological context dependent and shaped by earlier experiences through a learning component. Overall, the cognitive architecture of egg rejection decisions appears to be complex and shaped by the particular coevolutionary histories of hosts and parasites.
Introduction

The coevolutionary arms race between avian brood parasites and their hosts (Rothstein 1990) can be conceptualized as a war on two fronts that can be distinguished temporally: before and after the parasite lays its egg in the host nest. The first takes place prior to the insertion of the parasite egg and primarily comprises of the host’s attempts to prevent nest infiltration by the parasite (Welbergen and Davies 2009, Mclean 1987, see chap.19). The second pertains to host defense mechanisms following successful egg deposition by a parasite (egg, chick, and nestling rejection; nest desertion). At this stage, the generally (but not always: e.g., Servedio and Hauber 2006, Krüger 2011) most effective way to avoid investing parental care in unrelated offspring is the destruction, marginalization, or removal of the parasitic egg(s) prior to hatching. Accordingly, the identification and rejection of foreign eggs are among the most prevalent and also best studied adaptations to brood parasitism among common, parasitized species (Rothstein 1974, Brooke and Davies 1988, Feeney et al. 2014).

Whereas several physical and socio-ecological factors may play key roles in egg rejection behavior such as disruptions in the arrangement (“shuffling”) of eggs in a clutch (Polačiková et al. 2013, but see Hanley et al. 2015) and the observation of a nearby parasite individual by the host (Bártol et al. 2002), this chapter will focus strictly on the perceptual cognitive rules in assessing foreign eggs in the nest. Rothstein (1975) examined egg rejection under the context of the cognitive mechanism or decision “rule” that the host individual might use to determine which eggs from a parasitized clutch to
eject. He made reference to two candidate rules: the “odd egg out” or discrimination by discordancy mechanism, and the template-based “true recognition”.

Under the discordancy mechanism, hosts will selectively reject the egg(s) which are most dissimilar in appearance to others in the same clutch. This is perhaps the simpler of the cognitive rules, as the host’s discrimination task is not dependent on memory. Discordancy may seem to represent a reasonably successful strategy, as parasitic eggs are usually in the minority of any given parasitized clutch relative to the host’s own eggs (Hauber 2001). However, due to incidental variation in the eggshells of the host’s own clutch, this strategy may become highly error-prone (Lotem et al. 1995) and the host may accept foreign eggs or even reject its own egg(s). Own-egg rejection under the discordancy mechanism is especially likely in populations with such high parasitic pressure that multiple parasitism may place a host’s own egg in the minority of its clutch (Stevens et al. 2013). Nevertheless, the discordancy rule is a relatively parsimonious possible mechanism in part due to its direct nature, and during the early decades of egg rejection research it was accepted as the sole explanation for the behavior of rejecter species, as it lined up empirically with naturalistic field observation (but see below).

Under the template recognition mechanism, on the other hand, the host can discriminate a foreign egg by comparing its visual features to that of an innate or learned recognition template of the host’s own eggs’ appearance. This is considered ‘true recognition’ because it requires a cross reference to the host individual’s internal knowledge, and thus, a neural encoding of the template (Hauber and Sherman 2001).
Template based recognition can also explain diverse experimental patterns of parasitic egg rejection in cases of single parasitism where discordancy is evoked, but is typically rejected because it is less parsimonious (requires neural mechanisms for memory and internal comparisons); in turn, templates are specifically and singularly evoked to explain patterns of rejection, such as color dissimilarity-based increased rejection of foreign eggs from nests of multiply parasitized hosts where own eggs are in the minority or are absent altogether (Bán et al. 2013).

Fortunately, the logistics of experimentally teasing apart the relative prevalence of discordancy vs. template-based foreign-egg discrimination mechanisms in a given host population are relatively straightforward. For instance, a series of artificial parasitism experiments which vary in the relative makeup of host vs foreign eggs would have obvious predictions: hosts operating under the discordancy rule should reject the egg(s) which constitute the minority of the clutch even if they are its own eggs, whereas, conversely, hosts operating under the template recognition rule should preferentially reject foreign eggs, even when they represent the majority in the clutch. Many such experiments over the past 40 years have been carried out, resulting primarily in steadily mounting support for the template recognition hypothesis (Victoria 1972, Rothstein 1974, 1975, Moksnes 1992, Lotem et al. 1995, Sealy and Bazin 1995, Peer and Sealy 2001, Lahti and Lahti 2002, Lyon 2007, Strausberger and Rothstein 2009, Moskát et al. 2010, 2014b,c, Bán et al. 2013, Lang et al. 2014, Wang et al. 2015).
Support for Template Based Recognition

Victoria (1972) carried out 322 egg replacement trials on a captive colony of African village weaverbirds *Ploceus cucullatus*, which, in natural conditions, are commonly parasitized by the dideric cuckoo *Chrysococcyx cuprius*. The experiment demonstrated rejection behavior even in the absence of the host’s own eggs, and concluded that the weaverbirds possessed a relatively precise memory of the appearance of their own eggs, especially since rejection rate increased proportionally to the difference between the experimental egg and the egg it replaced. Following this, Rothstein (1975) demonstrated a similar capability in the gray catbird *Dumetella caroliniensis*. Moksnes (1992) carried out an experiment on chaffinch *Fringilla coelebs* and brambling *Fringilla montifringilla* nests in which all but 1 egg in a host’s clutch was replaced with a foreign egg. Under the condition wherein the experimental egg starkly contrasted the host’s natural eggshell, 10 out of 11 nests showed rejection of the foreign eggs, some going so far as to reject every egg until only the original host egg remained (these nests were ultimately abandoned by the host). Similarly, Peer and Sealy (2001) tested the same capability in the great-tailed grackle *Quiscalus mexicanus*, by swapping two eggs out of a clutch of three eggs with artificial eggs. In all 10 experimental nests, both artificial eggs were ejected even though the natural grackle egg which remained, had represented the discordant egg.

Lahti and Lahti (2002) followed up Victoria’s 1972 study on *P. cucullatus* with a rigorous field study of rejection rates in response to various levels of mimicry as well as relative proportion of host vs experimental eggs. The authors observed the rejection of the
single foreign egg in conditions wherein there were 2 host eggs, 1 host egg, and indeed zero host eggs. In fact, host subjects were not statistically better discriminators when they had their own eggs available in the clutch for comparison to the foreign egg. The authors point out that a template based rule would be particularly adaptive for *P. cucullatus* when compared to discordancy, as with an average clutch size of 2 eggs this species’ own eggs cannot be expected to maintain a majority in a parasitized clutch. Importantly, even though the study employed eggs with varying levels of mimicry, no distinct threshold of differential from own egg was found to elicit rejection universally, indicating individual variation in egg rejection decision making in a single population (see evidence for plastic threshold of discrimination below). This study, along with Victoria (1972) and Rothstein (1975) above, was one of many which explicitly exhibited host individuals’ ability to discriminate and reject foreign eggs even in the absence of one’s own eggs for comparison. Subsequently, the great reed warbler *Acrocephalus arundinaceous*, a host of the common cuckoo *Cuculus canorus*, has also demonstrated this ability with empirical rigor and through the manipulation of either egg maculation (Moskát et al. 2010) or shell background coloration (Bán et al. 2013).

**Evidence for Mixture of Discordancy and Template Based Rules**

None of the above is meant to imply that the discordancy mechanism does not exist in any host population, nor that discordancy and template based heuristics in egg rejection decision making are mutually exclusive, indeed there is mounting evidence that they are not
(Marchetti 2000, Moskát et al. 2010, 2014c, Stevens et al. 2013, Yang et al. 2014). One of the more compelling cases for the influence of a discordancy mechanism is the phenomenon of many host populations’ rejection rates reducing as the relative proportion of foreign eggs in the nest to own eggs increases (Moskát et al. 2009, 2010, Stevens et al. 2013). If template-based recognition were the only rule in place, hosts should be able to distinguish foreign eggs from their own just as easily no matter their propensity are in the clutch.

Recently, Yang et al. (2014) examined a population of ashy-throated parrotbill Paradoxornis alphonsianus, a host of the common cuckoo. This is an attractive host-brood parasite study system as P. alphonsianus individuals lay immaculate white, grey, or blue eggs, but only one color per female. The race, or gens, of C. canorus which parasitizes P. alphonsianus mimics the host’s eggshell pattern as is often the case with C. canorus (Brooke & Davies, 1988), but the authors in previous experiments found no indication that parasite individuals preferentially target hosts which lay the same color eggshell as they do (Yang et al. 2013). In this follow-up experiment, the researchers artificially parasitized nests with conspecific eggs not matching their own phenotype with variation in propensity of host vs foreign egg. Contrary to most studies detailed so far, this host population required at least one own egg in the nest for reference to reject foreign eggs; nests containing only manipulated eggs were accepted, indicating that rather than cross referencing a memory of its own eggs’ appearance, these individuals were employing “online-self phenotype referencing” (Hauber and Sherman 2001), rather than template-based discrimination, in real time when deciding whether or not to eject. In addition, some
individuals preferentially rejected their own eggs when they were in the minority, seemingly adhering to a discordancy rule. It is possible that this species’ lack of an internal template may be due in part to the immaculate nature of their eggshells, as maculation (spotting) has been theorized to play a critical cognitive role in the internalization of one’s own eggshell phenotype (Davies 2000, Stoddard et al. 2014).

Stevens et al. (2013) demonstrated how the discordancy component to the African tawny-flanked prinia Prinia subflava’s rejection strategy may be being exploited by the cuckoo finch Anomalospiza imberbis, of which it is the most common host. The authors first experimentally demonstrated that overall, hosts were consistently likely to reject a foreign egg across clutches with host:foreign egg ratios of 1:1, 1:2, and 1:3, showing strong evidence of a template-based mechanism in place. However, rejection rates decrease as the relative proportion of foreign eggs in the clutch increases, providing evidence for the influence of a discordancy rule (i.e., as the foreign eggs are becoming less discordant, rejection rates are decreasing). The authors go on to demonstrate that in the wild, individual A. imberbis parasite females will very often target the same host female twice, laying two eggs in the same nest. This, it seems, is a novel strategy which exploits the tendency for acceptance rates to increase as parasite egg count increases. However, the reported pattern of decreasing foreign egg-rejection rates across increasing multiple parasitism is also consistent with the mechanism that increased overall color and pattern variation of foreign eggs in the clutch induces a cognitive interference for template- or online self-referencing based discrimination mechanisms in that the foreign eggs represent a broader range of variation in multiple than single parasitism, thereby reducing the efficiency by which
recognition threshold based mechanism can discriminate own vs. foreign eggs (Hauber et al. 2006, Bán et al. 2013; see below). To fully assess these alternatives requires artificial egg introductions into host nests where the variability of the foreign eggs is experimentally controlled. Bán et al. 2013 conducted such an experiment partially, but a fully factorial study is still needed.

Earlier, Moskát et al. (2010) had conducted a similar experiment on the great reed warbler, host of the common cuckoo, demonstrating that the discordancy and template based rulesets could be experimentally disentangled and exhibited in a single population. The authors provided the test population with one of three nest setups: with one dyed egg and the rest unaltered, with all but one egg dyed, and with all eggs dyed. In the condition where the unmanipulated egg represented a minority, the host individuals rejected that egg (own egg) above control levels, indicating the influence of the discordancy rule. However, a full 33% of the nests which contained all manipulated eggs with no own eggshell pattern for reference matching showed rejection behavior, a clear sign of a template based ruleset. From this study it can be concluded that these rulesets can coexist in a single population and even influence individual behavior to each mechanism’s extreme (rejecting own minority egg due to discordancy and rejecting parasite eggs in the absence of any own eggs due to template-based matching).

Then Moskát et al. (2014c) investigated the phenomenon of rejection rates decreasing in cases of multiple parasitism from a perspective of variable clutch heterogeneity. The authors reasoned that as the number of foreign eggs in a nest increases,
the overall intraclutch variability will increase resulting in a disruption of discordancy-based decision making. They manipulated several great reed warbler nests with experimental eggs varying in color (and thereby extent of mimicry) and minority/majority status. They discovered that a host’s rejection rate of a given experimental egg color was largely contingent on the color of the surrounding eggs in the clutch, and concluded that discordancy-based egg rejection decisions are context dependent. Specifically, hosts seem to take into account the appearance of the entire clutch and the specific extents of mimicry both in the contextual minority and majority with regards to color. They termed this a mimicry-dependent discordancy rule, and its prevalence in other host-parasite systems still remains to be explored fully.

**Evidence for Plasticity of Discrimination Thresholds**

Regardless of which egg rejection decision rule is being employed, the cognitive task of a host deciding whether or not to eject an egg is essentially the same. Whether it is comparing the egg to an own egg in the nest, to an internal template of an own egg, or to the majority of the clutch, the host must make a decision (Moskát and Hauber 2007) regarding if the egg is different *enough* in comparison to putative own (majority) eggs to reject. Natural intraclutch variation is very common (Cherry et al. 2007) and can itself be variable from individual to individual (Lotem et al. 1995), so what constitutes “different enough” is not conceptually, empirically, and experimentally straightforward.
Lotem et al. (1995) examined rejection rates at a study site of the oriental reed warbler *Acrocephalus orientalis*, a host of the common cuckoo in Japan, in response to both artificial and natural parasitism. Coinciding with many other studies detailed in this chapter, they found that rejection rates were higher for experimental egg types most different in appearance to the hosts’ own. Interestingly, the egg types which were rejected less often took longer to reject, perhaps indicating a more effortful, difficult cognitive decision. These eggs, compared to the more often rejected extreme eggs, can be thought of as being closer to the threshold of acceptance, or the minimum amount of difference from template required to be perceived to execute rejection behavior, while the variable rates of rejection can be attributed to this threshold being unique to each host individual. The authors also discovered two important points regarding the mid-season, younger breeders in the host colony: 1) compared to the older females, their intraclutch variation in eggshell appearance was quite high and 2) their acceptance rate of foreign eggs was also relatively high. The authors reasoned that high intraclutch variation could result in higher collateral, own-egg rejection rates if they were prone to reject odd eggs, as any egg in a highly variable clutch may seem odd, and that this accounts for a lower overall rejection rate even of foreign eggs. Importantly, they posited that experience with their own eggs during their first breeding season might constitute a learning component for establishing the template for recognition and adjusting a special case of optimal threshold of acceptance (Reeve 1989) for subsequent breeding seasons.

This host age-dependent foreign egg rejection in the related great reed warblers was recently confirmed by Moskát et al. (2014a) who found that young, naïve breeders
are prone to accept experimental parasitic eggs both at the onset and the conclusion of egg laying, whereas older, experienced breeders are increasingly more likely to reject foreign eggs as egg laying progresses. Similar age-dependent patterns and increases in rates of egg-rejection were reported for individually known aged magpie *Pica pica* hosts of the great spotted cuckoo *Clamator glandarius* in Spain (Molina-Morales et al. 2014). In turn, Stokke et al. (2007) generated a model to predict changes in a host’s threshold of acceptance based on clutch characteristics. In keeping with the conclusions of Lotem et al. (1995), they assumed that experience with one’s own eggs during a host individual’s first breeding season is critical to the learning process, and that longer learning processes (template development and refinement) can be expected in hosts with greater intraclutch variation due again to the increased risk of recognition error and collateral own egg rejection. Finally, working on the North American catbird-cowbird system, Strausberger and Rothstein (2009) hypothesized that brood parasites might hijack this imprinting process by parasitizing first-time breeders, causing the hosts to develop their recognition templates on parasitic eggs and ultimately accept such eggs in the future. Indeed, the authors demonstrated experimentally that hosts of the brown-headed cowbird *Molothrus ater* decrease rates of rejection of foreign eggs in nests where the misimprinting event on a cowbird egg was artificially induced.

It appears that discrimination thresholds are not only adjusted between breeding seasons, mounting evidence indicates that such acute learning may be occurring over various stages in the egg laying cycle as well. Moskát and Hauber (2007) measured foreign egg rejection rates of the great reed warbler within one day just before egg laying
and during the egg laying cycle. They encountered a dramatic increase in rejection of common cuckoo eggs (28% to 75%) between pre-laying and after the first egg was laid (in this case, the parasite replaced the host egg). This appears to indicate that the innate template of egg appearance in the host was refined by the laying female’s own experience with her egg once it was laid, causing such a dramatic leap in the threshold of acceptance in mere hours. Additionally, rejection rates dropped back down as the laying cycle proceeded and the number (and, presumably, the variability) of host eggs in the nest following parasitism increased, showcasing several major shifts in the acceptance threshold of host individuals in this population over a single egg laying bout. Wang et al. (2015) found similar rejection rate variation in the yellow-bellied prinia *Prinia flaviventris* across several stages of a single egg laying cycle, with further variation based on extent of mimicry.

A host’s threshold of foreign-egg rejection is not only modulated by experience with its own eggs, it can also demonstrate plasticity as a result of experience rejecting a foreign egg. Honza et al. (2007) parasitized Eurasian blackcap *Sylvia atricapilla* nests by repeating experimental parasitism events on the same individual to gauge how rejection/acceptance response compared to a second attempt. They found not only that acceptors/rejecters were consistent in their decision between trials, but that those individuals which rejected during the first trial were *quicker* (showed a shorter latency) to reject during the second trial, indicating acute learning. Hauber et al. (2006) demonstrated perhaps the clearest example of experience-dependent threshold of acceptance modulation for a rejecter species. They demonstrate that the great reed
warbler will typically accept a near-mimetic egg with slightly altered maculation and will reject a more drastically altered egg which has had its background color completely masked. This should come as no surprise by now: hosts are more likely to reject a foreign or experimentally manipulated own egg as the less mimetic it becomes (Hauber et al. 2015). However, what the authors discovered was that individuals which had previously rejected the more drastically altered egg would also, in the same egg laying cycle, typically reject the near-mimetic egg which was accepted by the naïve individuals. The authors account for this acute modulation of the individual’s entire decision making heuristic by pointing out the original, extremely manipulated egg acted as a sign of parasitic threat in the area, likely prompting an increase in the attention and accuracy, and the narrowing of the thresholds of the various defense mechanisms and cognitive decision rules of the affected host individuals.

Concluding Remarks and Future Directions

Avian brood parasitism is a truly remarkable model system for coevolution; and cognitive adaptations by hosts to reject foreign eggs in response to the pattern and strength of parasitism are clear predictions of such an arms race. So rarely can we find such an intimate, one to one relationship between parasite and host such that responsive and compensatory traits can be so readily attributed to each other’s advances. Egg rejection in particular represents a phenomenal driving force in this increasingly complex game state. It has driven the common cuckoo to the seemingly herculean feat of host-specific eggshell mimicry which in turn may have driven host species to develop
more sophisticated cognitive faculties and perhaps even something resembling self-knowledge.

There should be no doubt now that the two cognitive rules thought to dictate egg rejection decision making are not necessarily mutually exclusive on the species, population, or even individual level. Clever experimental designs have disentangled them to show us they were entangled in the first place, and now variable levels of each can be measured in various species of various locations to get a clearer picture of the ever morphing shape of egg rejection behavior worldwide, and how it is a product of contextual parasite-host dynamics. The final decision to eject an egg or not is reliant on such variables as relative cognitive ability of the host, characteristics of the clutch, experience, and relative parasitic pressure. There are host populations under low pressure where multiple parasitism is rare, and so discordancy mechanisms have a relatively powerful influence. As well, the lack of maculation on their eggshells may account for their poor recognition template, which would in turn strengthen the case for maculation as a critical feature in functional templates of other species. In contrast, populations of hosts exist under high pressure and with a small clutch size, such that the discordancy rule would be highly maladaptive, and as such template recognition is strong enough to reject foreign eggs in the absence of one’s own. In these ways, the relative prevalence of egg rejection rulesets can, when measured rigorously, open new frames of reference for brood parasite dynamics on a global scale.
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Works Cited


Chapter 2: Does the house sparrow *Passer domesticus* represent a global model species for egg rejection behavior?

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Does the House Sparrow *Passer domesticus* represent a
global model species for egg rejection behavior?

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Abstract

Conspecific brood parasitism (CP) is a facultative breeding tactic whereby females lay their eggs in the nests of conspecifics. In some species, potential host individuals have evolved the ability to identify and reject foreign eggs from their nest. Previous studies suggest that the ubiquitous House Sparrow *Passer domesticus* in Spain and South Africa employs both CP and parasitic egg rejection, while a population in China does not. Given the species’ invasive range expansions, the House Sparrow represents a potentially excellent global model system for egg rejection across variable ecological conditions. The present study examines House Sparrow responses to experimental parasitism at three geographically distinct locations (in Israel, North America, and New Zealand) to provide a robust test of how general the findings of the previous studies are. In all three geographic regions egg rejection rates were negligible and not statistically different from background rates of disappearance of control eggs, suggesting that the House Sparrow is not a suitable model species for egg rejection experiments on a global scale.

Key words: brood parasitism, citizen science, invasion, metareplication
Significance Statement

House Sparrows (*Passer domesticus*) are the most geographically widespread bird on Earth. As such, they may represent excellent global models of organismal biology and ecology allowing comparisons of traits across varied ecological contexts. The identification and rejection of foreign eggs from one's nest is a central behavior of interest in the study of avian brood parasitism, and some studies out of Europe and South Africa seem to indicate that the House Sparrow engages in this behavior in response to being parasitized by conspecifics. However, a study from China reports a lack of this behavior in a native population, and our data reported in this study demonstrates a similar lack of this behavior in House Sparrows across three continents. This calls into question the external validity of egg rejection studies focusing on House Sparrows, and suggests that they do not represent a suitable global model species of this behavior.
**Introduction**

Avian brood parasitism incurs significant fitness costs on the host and is a strong selective pressure favoring the evolution of antiparasitic defensive strategies (Davies and Brooke 1989; Begum et al. 2012). One of the most common and effective countermeasures that hosts employ is the recognition and rejection of foreign eggs, usually by detecting differences in eggshell coloration and maculation between her own eggs and foreign eggs (Stoddard and Stevens 2010; Hauber et al. 2015).

In addition to interspecific brood parasitism (IP), studied most extensively in Common Cuckoos *Cuculus canorus*, conspecific brood parasitism (CP) is a facultative strategy in which females lay their eggs in conspecific nests (Davies, 2000). CP offers several key advantages to parasites over interspecific parasitism, including ease of nest access and ‘instant’ egg mimicry, and has been reported in over 250 bird species (Lyon and Eadie 2008). Counter-adaptations to CP are rare, as the naturally ‘mimetic' conspecific eggshells may represent a perceptual difficulty in foreign egg discrimination. Also, CP typically inflicts less of a fitness cost on the host than some obligate, interspecific brood parasites: while a host in an instance of CP pays a cost of caring for a typically unrelated individual, a host in an instance of IP suffers the same cost as well as the potentially higher cost of the death of its own chicks (e.g. via eviction of host progeny by obligate parasitic hatchlings; Grim et al. 2009). Yet, some hosts have evolved a fine-tuned discrimination ability even against very similar conspecific eggs, including nest desertion and selective egg rejection. (Lyon 2003, Samas et al. 2014). Nonetheless, experimental studies of CP
remain rare, especially compared to a massive research effort invested into the study of IP (Grim 2007).

One species that has been shown to employ CP is the globally widespread House Sparrow *Passer domesticus* (Kendra et al. 1988). Native to Europe and parts of Asia, the House Sparrow has a long history of introductions and invasions on all continents except Antarctica. Its contemporary cumulative native and introduced range renders it the most widely distributed bird on Earth (Anderson 2006). Therefore, the species represents an opportunity to study parasite-host dynamics and behavioral responses over vastly variable environmental and social conditions. In fact, the House Sparrow could represent a model species for studying global egg rejection behavior, given the ubiquity of the species; however, conflicting results found in the literature call such suitability into question (see below).

Here we examined whether House Sparrows are indeed a suitable ecological and experimental model for egg rejection studies by comparing published data on foreign egg rejection patterns with our new data from three geographically distinct populations. There have been several studies of egg rejection behavior in wild and captive populations of House Sparrows, focusing on individuals from either native or introduced populations in the wild or in captivity. An introduced population on Dassen Island, South Africa subjected to artificial parasitism (wherein a foreign conspecific egg was introduced to nests by an experimenter either in addition to existing eggs or by replacing an existing egg) exhibited a relatively high rate of foreign egg rejection (24-46%). Rejection in this case also proved to
be costly to the host, as 4 out of 9 cases of foreign egg rejection resulted in the ejection and destruction of the host’s own egg(s) (Lopez-de-Hierro and Ryan 2008).

Two more recent reports on a captive study population in Granada, Spain showed similarly high rejection rates in artificially parasitized Sparrow nests (27-33% and 44% of foreign eggs rejected, respectively; López-de-Hierro and Moreno-Rueda 2010; Soler et al. 2011). The 2010 study in particular provided data on differential host responses to treatments of the introduced egg, either variable size or manipulation of the shell color/spot pattern. Introduced eggs with variable brown spot patterns (i.e. "mimetic" eggs with manipulated maculation) produced a significantly higher rejection rate than those that were of a different color. In contrast, Soler et al. (2012) also in Granada, Spain, reported a baseline rejection rate of 30% for artificially colored (red) eggs but a 0% rejection rate for spotted eggs in a corresponding experimental design (see: ‘experimental group 2’ vs baseline experiment in Soler et al. 2012).

Lopez-de-Hierro and Moreno-Ruedo (2010) make reference to an artificial parasitism study on a wild introduced North American population of House Sparrows by Kendra et al (1988), citing a 35% rejection rate comparable to their own reported findings. However, a reading of the 1988 study reveals rather that 35% of individuals responded in some way to the experimental manipulation, most by skipping an egg-laying cycle and, in fact, only one individual (6%) was reported to actively eject eggs from her nest (Kendra et al. 1988).
Many other populations of House Sparrows in distant ecosystems, both native and introduced and both in captivity and in the wild, have yet to be studied regarding the propensity of CPB and egg rejection. A recent study by Yang et al. (2015) represents the first examination of a native Asian population in this regard, subjecting a freely breeding population in Northwestern China to artificial parasitism. Here, the researchers placed either clay model eggs or painted natural Sparrow eggs (either completely red or spotted with brown blotches) into nests within newly laid clutches, expecting rejection rates resembling the 2010 Granada study to which their protocol was comparable. They found a total absence of rejection behavior regardless of the type of experimental manipulation. These authors speculated that this discrepancy could potentially be attributed to an evolutionary equilibrium by which their study population had lost rejection behavior to avoid the high cost of collateral own-egg loss demonstrated in the European and South African studies.

Within any host of avian brood parasites there might be spatial and temporal variations in particular biological traits, including egg rejection rate; indeed, such geographic variation was detected in almost all studies that examined multiple host populations in the context of brood parasitism and egg rejection (e.g. Soler et al. 1999; Stokke et al. 2008; Polačiková and Grim 2010; Grim et al. 2011; Samas et al. 2014). Thus, the puzzle of whether House Sparrows are consistent egg rejecters is far from solved and more work is required to examine geographic, including native/introduced, and ecological, including wild/captive, differences in host-parasite dynamics in this ubiquitous species (Grim and Stokke 2016).
A critical way to establish the generality of patterns in biology is metareplication, i.e. repeating of whole studies across time, space and phylogeny (theory: Johnson 2002; empirical examples: Davies and Brooke 1989; Moksnes et al. 1991; Lahti 2005; Polačiková and Grim 2010; Grim et al. 2011; Samas et al. 2014). Another fundamental aspect of biological study design is the biological and statistical independence of the samples (Hurlbert 1984). Thus, we combine evidence from three new studies from three widely separated geographic regions of the world. This was made possible by previous human-assisted introductions of House Sparrows. Such model systems and species may be ideal for studying fundamental coevolutionary questions that are impossible to address in native host ranges (see Grim and Stokke 2016). Here we mirrored the protocols of Lopez-de-Hierro and Moreno-Ruedo (2010) and Yang et al. (2015) to experimentally test egg rejection among House Sparrows breeding in wild in the continental USA and New Zealand and in captivity in Israel. We then examined our data in relation to prior studies to assess the relationship of population history (native/introduced) and breeding type (wild/captive) upon egg rejection prevalence and the suitability of the House Sparrow species as a global model species of egg rejection behavior.

**Methods**

*Israel study*
Experiments were conducted on the House Sparrow colony in the zoological gardens of Tel-Aviv University (for more details, see Grodzinski et al. 2009), where individuals were kept in aviaries. The birds were provided with nest boxes, nest-building materials, sand for dust baths, mealworms *Tenebrio molitor* and ad libitum with water, seed mix and dry insect mash. The study was carried out during the breeding season of 2010. Nest boxes were monitored on a daily basis, information on the nest-building process, laying date, clutch size and evidence for possible instances of CP was gathered. As the House Sparrow lays one egg every 24 h, background levels of CP were inferred if there was an appearance of two new eggs at the same day (Yom-Tov 1980).

For the manipulation, the experimental eggs (n = 38) were inserted into host nests during the third or fourth morning of egg laying (i.e. after the host has laid its third or fourth egg). Each nest was artificially parasitized once and eggs were introduced both via the egg exchange method (exchanging one of the eggs with an experimental egg, n = 21) and the egg introduction method (adding an experimental egg to the clutch, n = 17). The experimental eggs were fresh natural conspecific eggs taken from other nests in the same colony. Furthermore, since various studies have shown that acceptance and rejection of the parasitic egg may vary throughout the course of the breeding season (Lotem et al. 1992; Lotem et al. 1995; Brooke et al. 1998), the experimental eggs were introduced throughout the breeding season.

Natural sparrow eggs were modified to form three different variants of foreign, "parasitic" eggs (Fig. 2.1): dark brown painted eggs (n = 6), light brown painted eggs (n =
and natural eggs with painted dark brown spots (n = 21). The egg features were modified using an acrylic non-toxic paint (Decoart ©-Asphaltum DA180 and Cool White DA240). Seven unpainted eggs were also used (and never rejected), but were removed from analysis to allow comparisons with the other datasets from USA and New Zealand where such eggs were not used. After the introduction of the parasitic egg, nests were checked for rejection on a daily basis. Rejection was noted when the foreign egg was missing from the nest ("ejection") or was found buried under nesting material ("burial"; López de Hierro and Moreno-Rueda 2010). An egg was considered "accepted" if it remained in the nest at least for 6 days after the start of the experiment (Lotem et al. 1995). Nests were also monitored in order to determine hatching and breeding success, thus in practice we could also detect egg rejection had it occurred after the six days criterion (which did not happen).

**Continental USA study**

In 2013, we originated The House Sparrow Project, a citizen science program in which volunteers who regularly monitor birds nesting in boxes across the lower continental USA, North America, carried out a standardized experimental protocol with House Sparrow eggs (for more details, see Larson et al. 2015). Volunteers reported on 114 House Sparrow nests in 16 different geographic localities. For a given nest, participants began the experiment when at least three eggs were already laid. Volunteers labeled each egg with a number at the sharp pole, and carried out one of three randomly assigned treatments: all brown, all red, or 20 brown spots. In the all brown and all red treatments, one egg was chosen at
random and painted completely using a felt-tip marker pen (Prismacolor™). In the 20 brown spots treatment, one egg was chosen at random and painted with twenty brown spots, each approximately 5 mm in diameter (Fig. 2.2).

As a "manipulated control", in addition to one of the experimental treatments, one egg (other than the experimental egg) was chosen and painted all over with a Prismacolor™ Premier colorless blender, which is a pen type containing the solvent found in the other two pen types, but not the pigment. Eggs handled in the same way as manipulated eggs (experimental and control) but not painted served as "unmanipulated controls".

An egg was considered accepted if it was present in an active nest for at least 6 days after the treatment was applied; this threshold has been used in the study of both interspecific (Lotem et al. 1995) and conspecific parasitism (Samas et al. 2014). Nests were monitored every 1-2 days until eggs were considered accepted or rejected. An egg was considered rejected if the egg went missing from the nest. At each monitoring visit, all eggs were removed, and manually checked for possible signs of breakage and then returned to the nest. Any new eggs were numbered, and any egg losses were noted. Eggs that were broken by the experimenter, and eggs in nests that were abandoned or depredated were removed from the analyses.

New Zealand study
Experiments took place between November 2012 and January 2013 in the carpark of the Miranda Naturalist's Trust on the Firth of Thames, North Island, New Zealand. Sparrows nested in dense bushes and built free-standing nest structures, rather than in cavities, at this site. We located clutches (n = 40) in active nests (n = 31) by visually searching and following adults carrying nesting material; some nests had multiple successive clutches laid in them. A new clutch was deemed to have been started if the nest was found empty between two visits where it contained eggs, or if all marked eggs were absent from a nest, and replaced with new eggs.

Three egg treatments were used, and randomly selected for each clutch. In the all-brown treatment, one egg was chosen and painted completely brown using a Sharpie™ brown marker pen. Other eggs in the same clutch were numbered at the sharp pole using a marker to allow identification of individual eggs. In the 20-spot treatment, one egg was chosen and painted with 20 brown spots with the same color marker as above. Other eggs in the clutch were numbered as in the all brown treatment. In the control treatment, one egg was chosen and painted all over with an Artline™ colorless blender, which is a pen containing the solvent but not the pigments found in the other pens. The chosen egg and all others in the clutch were numbered as in the all brown and 20-spot treatments.

In nests with the clutch completed (i.e. same number of eggs in two consecutive days), one egg was chosen at random to be the treated egg. For nests where laying was incomplete, some were selected at the time of discovery to have a subsequent egg be the experimentally treated egg in an attempt to keep the probable proportions of treated eggs
even with respect to laying order. Nests were monitored until nest failure or fledging of
chicks. At each monitoring visit, all eggs were removed from the nest. Any new eggs were
numbered, treated or both, and any egg losses were noted. Any eggs present in an active
nest for 6 or more days were deemed “accepted” (Lotem et al. 1995), and any egg lost from
the nest within that time was deemed “rejected.”

Statistical Analyses

For all experiments, a general linear model (GLM) was generated to determine any
difference between the rejection rates from our treatment groups. In this way we aimed to
determine whether the rejection of a manipulated egg differs from the background
disappearance of control eggs. Similar comparisons were also carried out comparing our
datasets to each other as well as to previously published reports from various authors and
countries. A logistic regression was used to compare previous studies with regard to
location, native/introduced status, and captivity, but colinearities between geographic and
ecological contexts caused the model to fail to fit; this is because there were no captive
studies conducted in the introduced regions of the species distribution.

Results

Israel Study
There were no instances of two eggs being laid on the same day; thus, no detectable cases of CBP were observed. Furthermore, out of the 31 nests that were artificially parasitized, no introduced eggs were rejected (i.e. eggs were retained regardless of treatment, Fig. 2.3a).

**Continental USA study**

No significant differences were detected between the experimental treatment types (all brown, all red, and 20 brown spots) (GLM: $F_{2,108} = 0.43, p = 0.65$, Fig. 2.3b). Overall, there were no significant differences in rejection rates between the experimental eggs (regardless of treatment), control eggs, and other eggs in the nest (GLM: $F_{2,326} = 0.32, p = 0.72$, Fig. 2.3c).

**New Zealand Study**

Overall, from 35 experimental nests, a total of 3 rejections (8.6%) occurred. There was no significant effect of treatment type (all brown, 20 brown spots, and control) on the rejection rate ($F_{2,32} = 0.05, p = 0.96$, fig. 2.3d).

**Combined Analyses**
There were no significant locality-based differences across our three datasets with regard to rejection rate of experimental eggs (GLM: $F_{2,174} = 1.37, p = 0.26$). Combining our data with those of Yang et al. (2015) also yielded no significant effect of dataset on egg rejection rate (GLM: $F_{3,218} = 2.23, p = 0.09$). However, studies in Spain and South Africa reported higher rejection rates (Table 1). Combining our data across all of the previous studies revealed a significant effect of the source of dataset on reported rejection rates of the experimental egg ($F_{8,546} = 9.659, p < 0.01$).

Discussion

House Sparrows in all of our three independent and geographically isolated study sites demonstrated very low or absent egg rejection. Furthermore, rejection rates did not statistically differ between experimental eggs and control eggs. This implies a lack of foreign-egg discrimination behavior in our distinct House Sparrow study populations. The highest rejection rate here was 11.4% for the all brown treatment eggs in the continental USA study, but even this rate was much lower the 27-35% reported in the 2010 Granada study (López de Hierro and Moreno-Rueda 2010) and statistically not different from the background pattern of egg disappearance of control eggs across the continental USA population. In fact, whereas previous authors found the strongest rejection response to manipulating the maculation pattern of eggs artificially, our continental USA dataset’s lowest rejection rate was in the 20-brown-spot treatment group (5.4%). Some published works on House Sparrow egg rejection behavior cited a 35% rejection rate from a 1988
study, yet in that study only one individual (6%) actively ejected foreign eggs from its nest (Kendra et al. 1988) according to the definition of egg rejection adopted in our study and in other studies we have reviewed (see Methods).

A conceivable explanation for the discrepancies between our data sets and the 2010 Granada study is that two of our populations are wild and introduced whereas the Granada population is captive and native. However, our Israel data were collected from a captive population within the native range of House Sparrows, thus it represents the most direct comparison with the Granada study, and yet it showed the lowest rejection rate of all three studies (complete absence). Relative breeding density and therefore risk of CP may be a contributing factor to anti-parasitic behavior (Samas et al. 2014), and indeed our Israel population was at a low breeding density (not all nest boxes were used and no instances of CP were detected), suggesting a possible explanation for the discrepancies between our Israel data and the Granada data sourced from a high density population with high rates of CP. Yet this argument is once again challenged by the high recognition/rejection rates out of the South African study, which appear to have occurred under low CP risk (López de Hierro and Ryan 2008).

Furthermore, the South African study shows high rates of discrimination and rejection behavior in a wild introduced population; whereas, our study showed little rejection in wild introduced populations in USA and New Zealand, also indicating the lack of a direct relationship between rejection behavior and introduced status. It is likely instead that the different statistical results of these studies and ours are a matter of methodology.
and definitions. For instance, in their study, López de Hierro and Moreno-Rueda (2010) considered a foreign egg "accepted" only when it remained in the nest until at least one egg in that brood hatched. All other circumstances that may lead to a brood failing to produce hatchlings were considered an identification and direct rejection of the foreign egg by the host female. In our studies, we focused only on selective egg ejection.

Yang et al. (2015) suggested the putative loss of rejection behavior in their observed population could be a response to relative cost of rejection, given the high instance of collateral own-egg loss during rejection behavior reported in the literature. Indeed, our USA and New Zealand data show no significant difference in rejection rate between foreign, control, and own eggs in the few cases where rejection did occur, suggesting a high reproductive cost of this behavior as it resulted in the equivalent destruction of related and parasitic offspring. However, the reported relationship between rejection rate and rejection cost is not consistent in the literature, as two studies on the same captive population in Granada produced two very different estimates of rejection costs, from almost half (44.4%) of own eggs destroyed to almost none (4.1%), and yet the reported foreign egg rejection rates were 26-30% and 44% respectively (see Moreno-Rueda and Soler 2001 vs. Soler et al. 2011 respectively).

Comparing the published data with our own from across Europe, Australasia, and Africa suggests two conclusions. First, no direct explanation exists regarding native/introduced status or captive/wild studies for why egg rejection behavior seems regionally restricted in House Sparrows. Second, in general, egg rejection behavior can
now be considered rare in most studied populations of Sparrows, thereby rendering this species unsuitable as a global model species for the evolution, development, and plasticity of antiparasitic defenses at the egg rejection stage.

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Conflict of Interest: All authors declare no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.
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Figure 2.1: Experimentally modified eggs used in the Israel study. A: dark color painted eggs, B: diluted color painted eggs, C: unpainted dark variant (removed from analysis), D: unpainted bright variant (removed from analysis), E and F: natural eggs with dark brown spots.
Figure 2.2: Eggs on color cards experimentally modified by volunteers in the House Sparrow Project across the continental US. a) The all red treatment (egg 3). b) The all brown treatment (egg 3). c) 20 brown spots treatment (bottom egg). In all images egg 2 was covered with a clear marker.
**Figure 2.3:** Host responses to experimental parasitism across global geographic scale: data from Israel (a), individual experimental treatments in the continental USA (b), overall data from the continental USA (c), and data from New Zealand (d). Sample sizes are displayed in each bar. For appearance of eggs used in various treatments see Figs. 2.1 and 2.2. For details see Methods.
Table 2.1: Experimental egg rejection rates with sample size, native vs. introduced status, and wild vs. captive status across various egg rejection studies of the house sparrow.

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<th>Captivity status</th>
<th>n</th>
<th>Rejection rate (％)</th>
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<td>Captive</td>
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Chapter 3: Multiple parasitism reduces egg rejection in the host (*Acrocephalus arundinaceus*) of a mimetic avian brood parasite (*Cuculus canorus*)

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Multiple parasitism reduces egg rejection in the host

(*Acrocephalus arundinaceus*) of a mimetic avian brood parasite

(*Cuculus canorus*)

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Abstract

A host that has been targeted by an avian brood parasite can recover most of its potential fitness loss by ejecting the foreign egg(s) from its nest. The propensity for some hosts to engage in egg rejection behavior has put selective pressure on their parasites to evolve mimetic eggshells resembling the host’s own shell colors and maculation. In turn, hosts have counter-evolved increasingly more sophisticated detection methods such as narrowing visual egg acceptance thresholds or using social cues to recognize parasitism. However, multiple cognitive mechanisms acting simultaneously could theoretically interfere with one another and ultimately decrease egg rejection accuracy, especially if these heuristics yield differing targets for rejection. By painting hosts own eggs, we studied a host species of the common cuckoo *Cuculus canorus*, the great reed warbler *Acrocephalus arundinaceus*, and tested its responses to the presence of “foreign” eggs of varying quantity, colors, and uniformity. Using reflectance spectra of egg background coloration and avian perceptual modeling, we then estimated the sensory thresholds triggering egg rejection by this host for each treatment. As previously reported, rejection rates were positively related to the perceptual distance between own and foreign eggs in the nests in all treatments. However, rejection thresholds were more permissive (error prone) both with greater proportions of foreign eggs per clutch and/or when the suite of foreign eggs was perceptually more variable within the nest. These results suggest that parasites, through multiple parasitism, can partially overcome the evolution of hosts’ recognition of mimetic parasite eggs.
**Key words:** acceptance thresholds; coevolution; host-parasite interactions; sensory modeling.
Hosts of obligate avian brood parasites incur fitness costs by providing parental care for genetically unrelated parasitic offspring, often at the expense of some or all of their own brood; the resulting selective pressure has caused the evolution of various sensory and cognitive abilities in hosts to defend themselves against parasitism (Davies 2000, Soler 2018). Among these, the rejection of foreign eggs, before they hatch, represents a critically important defense strategy partly because many parasitic hatchlings may eject or outcompete host eggs and chicks, leaving the host parents to exclusively rear the foreign chick (Feeney et al. 2012). A coevolutionary arms race may then proceed such that parasites respond to hosts’ egg rejection by laying increasingly more mimetic eggs, which more closely resemble the host eggs both in color and maculation (Yang et al. 2010; Stoddard and Stevens 2011; Geltsh et al. 2017). In turn, hosts might adopt more finely tuned egg perceptual discrimination strategies against imperfectly mimetic eggs (Pozgayová et al. 2011; Stoddard et al. 2014).

At least three non-exclusive cognitive mechanisms underlying foreign-egg recognition have been identified in hosts: discordancy, online self-referencing, and template-based recognition (Manna et al. 2018). Under the discordancy mechanism, hosts assess and reject the egg that has the most dissimilar appearance in the clutch (Rothstein 1974). Self-referencing, in turn, takes place after a host has laid its own egg(s) and learned to identify them, allowing for effective comparison to other (foreign) eggs also present
within a clutch (Hauber and Sherman 2001). Finally, template-based recognition relies on an internal (learned or genetic) model of host egg characteristics such that foreign eggs can be identified even in the total absence of the host’s own eggs in the clutch (Bán et al. 2013). The integration of these mechanisms is predicted to produce more accurate rejection decisions at the time of egg recognition (Moskát et al. 2010), especially in a host population with a high frequency of multiple parasitism (Stevens et al. 2013), where the host’s own eggs may be outnumbered or altogether replaced by parasitic eggs (Moskát et al. 2009).

Yet, empirical and experimental evidence suggests that when facing multiple parasitic eggs in the nest from one or more parasite females, hosts of mimetic brood parasites relax, instead of tighten, their recognition thresholds and reject fewer parasitic eggs, relative to single parasitism (Moskát et al. 2009; Bán et al. 2013; Stevens et al. 2013, Gloag et al. 2014). In contrast, hosts of non-mimetic brood parasites do not alter their rejection rates in response to varying proportions of parasitic eggs in the nest (Lang et al. 2014; Croston and Hauber 2015). This phenomenon of reduced rejection rates of mimetic parasite eggs in multiple parasitism could be due to perceptual interference caused when multiple cognitive mechanisms yield incongruent targets for egg rejection in the same clutch. For one example, if foreign eggs constituted the majority of the clutch, discordancy and template-based matching would yield opposite targets for rejection: own and foreign eggs, respectively.

Here we report on a set of experiments for which we manipulated both the proportion of foreign eggs in the nest and/or the variability of those foreign eggs to assess
whether and how each of these sensory cues might affect the accuracy of egg-rejection decisions by hosts of a mimetic brood parasite. Critically, our studies represent an experimental improvement, as previous studies examining the impact of multiple parasitism on rejection rates by hosts of mimetic parasites did not manipulate these two factors fully independently of each other (e.g., Moskát et al. 2009; Bán et al. 2013; Stevens et al. 2014).

In each of the experiments below, we studied egg rejection behaviors by the great reed warbler *Acrocephalus arundinaceus*, which is a heavily parasitized host of the cuckoo *Cuculus canorus* in Hungary (Moskát and Honza 2002) and experiences high rates of multiple parasitism (>50%, Moskát et al. 2009). Behavioral studies of this species at our study site suggest the use of either discordancy (Cherry et al. 2007; Moskát et al. 2009, 2014a) or template-based (Moskát and Hauber, 2007; Bán et al. 2013) foreign-egg recognition. In turn, Moskát et al. (2010) provided evidence for both discordancy and template matching in this population by varying the spot density of host’s own eggs and number of manipulated eggs in clutches. Increasing the variability of egg appearance in nests resulted in reduced rejection rates of foreign eggs from those nests (Moskát et al. 2008), although in that study the relative proportion of own eggs in the nest was not manipulated.

To identify foreign eggs in the nests, hosts have been thought to use perceivable dissimilarities between the appearance of the eggs in its nest such as background color and maculation patterns as cues (e.g. Cherry et al. 2007; Avilés et al. 2010; Stoddard and Stevens 2011; Stevens et al. 2013; Croston and Hauber 2014; Hauber et al. 2015;
Stoddard and Hauber 2017 but see Hanley et al. 2017). To quantify avian perceptual egg mimicry especially regarding eggshell background coloration, reflectance spectra in the avian visible range should be collected, followed by the application of visual modeling of avian tetrachromacy (Avilés 2008; Cassey et al. 2008).

Here we used shell coloration reflectance spectra and avian perceptual visual modeling to investigate the causal relationship between the presence of one or multiple foreign eggs of the same or varying artificial colors in the nest and the frequency of accurate egg rejections by great reed warblers. We predicted that the avian perceptual distances in shell background coloration between painted and natural host eggs would be positively related to the behavioral egg rejection rates in both single and multiple parasitism. We also predicted that the slope of the correlation between self-foreign egg difference and rejection rate should decrease in multiple parasitism, leading to more permissive egg rejection thresholds. A critical, and previously untested, third prediction pertains to the variability of foreign eggs in a multiply parasitized nest. Specifically, if the interference of cognitive rules reduces accurate egg rejection rates in multiple versus single parasitism, we should expect experimental increases in the color variability of the foreign eggs to further decrease the accurate rejection rates of foreign eggs in the nest.

To these aims, we compared the behavioral responses to experimental parasitism for which the foreign eggs were singly or multiply introduced, with either homogenous or heterogeneous coloration. If these mechanisms are indeed producing contradictory instructions, and if this dissonance does interfere with the host’s discrimination ability, we
can expect the accuracy of parasitic egg rejection to decrease as the relative proportion of foreign eggs increases in the nest. Furthermore, we predict that as multiple foreign eggs in a host clutch are more chromatically variable, the accuracy of foreign egg rejection will further decrease.

**Methods**

**Experimental Parasitism**

The data used for the current set of analyses were derived from published field experiments conducted in the breeding seasons from mid-May to mid-June in 2000 to 2001 (Bártol et al. 2002: treatment a, with natural cuckoo eggs), in 2003 to 2005 (Hauber et al. 2015: treatment a, with natural conspecific host eggs), and in 2009, 2010 and 2012 (Bán et al. 2013 treatment a, with painted own eggs; and treatments b, d, and e), and were supplemented with new, unpublished data collected by us in 2013 (treatment c). Critically, the between-treatment comparisons of the perceptual modelling outputs for both existing and new data are also entirely novel.

The study subjects were a population of great reed warblers inhabiting central Hungary. In this study area, great reed warblers build nests in reeds along both sides of water channels and usually have five eggs in a clutch (Moskát and Hauber 2007). Here, cuckoos typically remove one host egg when they lay an egg, and they appear to choose an egg randomly from the clutch, either a host or a cuckoo egg, if the nest has both egg types
(Moskát and Honza 2002). For this reason we retained clutch size to be constant in our experiments following natural parasitism.

We sought out nests in the building or egg laying stage and included them in the experiments. On the day of laying the 4th or 5th egg in a clutch, the background color of 1, 3 or 5 of the host’s own egg(s) was manipulated using Stabilo Boss™ highlighter pens. One of the following egg-painting treatments was assigned to each clutch (Fig. 3.1): treatment a: 1 egg in a clutch was painted blue, green, red, yellow, or orange, or replaced by a natural cuckoo or con-specific host egg (depending on the published field experiment from which the data were derived); treatment b: 3 eggs in a clutch were individually painted one color each (blue, yellow, and orange); treatment c: 3 eggs in a clutch were all painted the same single color (blue, yellow, or orange); treatment d: all eggs in a clutch were individually painted one color each (blue, green, red, yellow, and orange); treatment e: all eggs were painted the same single color (blue, yellow, or orange).

To control for the various treatments, unmanipulated eggs in the same clutches were also handled during the application of the treatments. Experimental nests were monitored for 6 consecutive days, and foreign eggs were considered accepted if the eggs remained in the nests on the 6th day. Eggs missing between subsequent visits were considered ejected by the host female. We analyzed the data on a per nest basis in each treatment, and so if one or more experimental eggs were missing before the 6th day, we termed that outcome to be rejection for that nest. To identify rejection by nest desertion, we monitored the temperature of eggs in experimental clutches. If eggs were found to be cold (i.e., same as ambient
temperature), we positioned the eggs with the sharp poles oriented towards the center of the nest so we would be able to easily detect egg rotation by the female in our absence (Bán et al. 2013.) If the eggs were still cold and this configuration remained unchanged after one day, we termed that outcome to be desertion.

**Quantifying perceptual differences between foreign eggs and host eggs**

Reflectance spectrometry data from each of the natural and experimental egg types and colors were collected for the full avian visible spectrum (300-700 nm) and sourced from Bán et al. (2013). Avian visual modeling was applied to calculate perceptual thresholds as chromatic just noticeable differences (JNDs) by Avicol v6 (Gomez 2006) between foreign eggs and natural host eggs. Since the visual sensory physiological data for the great reed warbler are not available, we used physiological parameters of the visual system of another rare cuckoo host, the European blackbird, *Turdus merula*, to characterize sensory thresholds of the avian visual system. This egg-rejecter species has been used repeatedly in avian reflectance spectrometry in the context of brood parasitic egg rejection (Igic et al. 2012, Croston and Hauber 2014), and indeed most UV-sensitive songbirds have similar visual systems (Hart et al. 2000, Aidala et al. 2012). Nonetheless, relying on other species for a focal host’s physiological data remains a limitation of visual modeling approaches at this time.

We used published ambient light irradiance spectra representing a broken canopy forest (Vorobyev and Osorio 1998), which coincides with the light condition of warbler
nests in reed beds (following Igić et al. 2012; Moskát et al. 2014a; Hauber et al. 2015). We applied a Weber fraction of 0.1 and the ultraviolet-sensitive visual model which is appropriate for thrush-like passerines (Aidala et al. 2012). The relative cone densities used for each photoreceptor type were 1 (ultra-violet sensitive: UVS), 1.78 (short wavelength sensitive: SWS), 2.21 (medium wavelength sensitive: MSW), and 1.96 (long wavelength sensitive: LWS) (Hart et al. 2000). Because we did not collect reflectance data on host eggs from each experimental nest, we randomly paired the spectral reflectance of foreign or painted eggs with the reflectance data of natural host eggs. In AVICOL we generated the chromatic and achromatic contrasts (JND) between foreign/painted eggs and host eggs. Achromatic contrasts (differences not relating to color) between foreign and host eggs were not significantly related to rejection rates in any of the treatments b - d (data not shown), and were omitted from the rest of the analyses.

Because none of the egg types were rejected at the limits (0 or 100%) of the range of possible rejections, following Hauber et al. (2015), we applied linear regression analyses to examine the potential relationship between the chromatic contrasts (JNDs) and rejection rates of differently colored eggs and calculated the JND values at which 50% of the foreign/painted eggs would be predicted to be rejected from the nest within each treatment (JND₅₀%). Lower JND₅₀% values represent more restrictive acceptance thresholds and, thus, fewer acceptance errors of foreign eggs in the clutch (Reeve, 1989; Stevens et al. 2013).

**Results**
The chromatic perceptual differences (JNDs) between foreign eggs and host eggs were positively correlated with the rejection rate in all treatments (all \( P < 0.1 \)) and significantly \( (P < 0.05) \) in treatment a, treatment b, and treatment e even, when restricting the analyses to blue (B), yellow (Y), and orange (O) eggs only (to keep color diversity comparable across treatments, Table 3.1).

Regarding the slopes of these relationships (Fig. 3.3), and the resulting JND\(_{50}\%), we found that multiple parasitism resulted in higher JND\(_{50}\% \) values (i.e. less strict acceptance thresholds) than single parasitism, and that within corresponding proportions of foreign eggs in the clutch (3/5 vs. 5/5 eggs), more variable multiple parasitism yielded higher JND\(_{50}\% \) thresholds than multiple parasitism by the same foreign egg color (Table 3.1).

The resulting order JND\(_{50}\% \) values showed a pattern of treatment a < treatment e < treatment c < treatment d < treatment b, which supports our critical predictions of treatment a < all other treatments, treatment c < treatment b and e, and treatment e < treatment d. Assuming that JND\(_{50}\% \) values between two treatments are never exactly the same and, thus can be either greater (>) or smaller (<) than JND\(_{50}\% \) values in any other treatment each with a 50% chance, the binomial statistical likelihood of our predictions to hold up simultaneously as seen in the actual data is \( p = 0.025 \).

Discussion
These results support our hypotheses that (i) increasing perceptual differences between hosts’ own egg background colors and foreign eggs in the nest resulted in increased rejection rates in great reed warbler hosts, (ii) this positive relationship weakened in instances of multiple parasitism, and (iii) within corresponding proportions of multiple parasitism (3/5 vs 5/5 eggs), more variable foreign egg colors in the nest exhibited a further decrease of the strength between perceptual distance and rejection rate.

Our findings of consistently positive correlations between perceptual distance and rejection rate corroborate the results of previous studies examining human assessment of color differences between host and parasite eggs (e.g., Honza et al 2004), physical metrics of egg color reflectance spectra distances (e.g., Cherry et al. 2007; Cherry and Bennett 2001; Takasu et al. 2009), and avian perceptual modeling (e.g., Avilés et al. 2010; Cassey et al. 2008; Stoddard and Steven 2011; Stevens et al. 2013, Stoddard et al. 2014, Hauber et al. 2015 but see Hanley et al. 2017).

Identifying which cognitive process(es) may be employed by a rejecting host can be difficult (Manna et al. 2018). This is because foreign egg acceptance does not necessarily imply a lack of cognitive processing (i.e., recognition without rejection: Moskát and Hauber 2007), and the rejection (or acceptance) targets of two or more cognitive mechanisms can overlap. In the great reed warbler, the main mechanism for egg recognition is probably template recognition, which might be further impacted by clutch-specific discordancy (Moskát et al. 2014a). Here we collected new experimental data specifically to complete a ‘missing treatment’ required for our broader analysis (treatment
c), and found that in this new data set the great reed warbler hosts rejected differently painted eggs at the same relative rejection rates (blue < yellow < orange) as was observed by Bán et al. (2013). However, our treatment c data alone cannot confirm the conclusion of Bán et al. (2013) that a learned template recognition mechanism can yield consistent egg rejection patterns across varying conditions of multiple parasitism. Nevertheless, these new data contradict the hypothesis that this host species may exclusively use discordancy-based egg rejection since the hosts’ own eggs were always in the minority of the clutch in treatment c (Figure 3.2). Overall, the novel analyses reported here confirm that using artificially colored eggs with various degrees of similarity to the natural host egg’s color can be useful in studying the perceptual basis of rejection behaviors by hosts (Hauber et al. 2015 vs. Lahti 2015).

Our findings show that multiple parasitism using both uniform and variable foreign eggs (treatments b-e) yielded more relaxed acceptance thresholds and, thus, more rejection errors, than single parasitism (treatment a). This suggests that great reed warbler hosts of their mimetic brood parasites have not evolved any fully reliable defense mechanism towards multiple parasitism. This could be because multiple parasitism is rare in most cuckoo hosts, including other populations of the great reed warbler (Wyllie 1981), and so their responses are adapted to brood parasitism with a single cuckoo egg. However, our study system is subject to unusually and consistently high levels of multiple parasitism, stable at least within the last century (Zölei et al. 2015), and so this host population is especially pressured to exhibit more sensitivity toward this particular recognition task and parasitism context. We therefore consider that template-based recognition is much more
effective for accurate cuckoo-egg recognition under these conditions of multiple parasitism than discordancy alone. However, the role of discordancy is also not yet fully understood, as the reduced egg rejection rates in multiply parasitized clutches suggest that discordancy effects do not improve upon, but rather hinder the hosts’ egg rejection abilities based on template recognition (Moskát et al. 2009, 2014a; Bán et al. 2013; present study). This finding in particular suggests a decrease in foreign egg discrimination accuracy resulting from the interference of simultaneously activated cognitive processes in this common cuckoo host.

We demonstrated an even further reduction in foreign egg rejection in clutches multiply parasitized by variably colored rather than uniformly colored eggs. This finding is in accordance with observations on natural, cuckoo parasitism in our study area. Specifically, here multiply parasitized nests are typically parasitized by different cuckoo females (Moskát et al. 2009), as different eggshell color and spotting patterns indicate that cuckoo eggs in the same nests were laid by different females as indicated by Moksnes et al. (2008) which supported the “constant egg-type appearance” hypothesis demonstrating that a single female’s egg appearance is constant across her own eggs. For this reason, our experiments on multiple parasitism represent the natural conditions of these host individuals in treatments b and d, but not in treatments c and e (homogenous multiple parasitism) which represent artificial contexts. Despite this, these homogenous multiple parasitism treatments are especially important to understand how recognition is driven perceptually. The reduced rejection rate under the variable vs. the homogenous foreign egg color conditions may be due to an even more disruptive activation of the discordancy
mechanism. That is, as each egg in the nest becomes unlike each other egg (and, therefore, a target of a discordancy-based decision), the template-based mechanism that would result in a successful rejection may become more suppressed by multiple simultaneous activations of the discordancy mechanism.

Although recognition mechanisms might be difficult to distinguish from a methodological point of view (see above), the present study demonstrated that the strength of positive correlation between egg appearance disparity and rejection rate can be weakened in multiple parasitism (also see Stevens et al. 2013). Consequently, hosts are less protected by accurate egg rejection in multiple parasitism than in single parasitism, which should be favorable for the parasite. However, in the case of the cuckoo, only one chick can be fledged from a nest as a consequence of the eviction behavior of young cuckoos (Wyllie 1981). The advantage of multiple parasitism for cuckoos is therefore limited, and there is a demonstrably small relative range of parameters when it could truly favor cuckoos engaging in multiple parasitism (Takasu and Moskát 2011).

Future studies will help illuminate the different cognitive mechanisms guiding the detection of foreign eggs by including additional colors (red, green; or a range egg coloration resembling natural egg color diversity: Hanley et al. 2017; Canniff et al. in press) that go beyond the current range of artificial painting treatments and using a range of naturally variable parasite eggs (Stevens et al. 2015, Lahti 2015). Whereas coloring the eggs of the host is a powerful means of experimentally isolating the effect of color on rejection (Hauber et al. 2015, but see Lahti 2015), future research could also examine great
reed warblers’ use of additional or complementary cues like egg shape, size, or speckling pattern (Cherry et al. 2007). It is possible that the cognitive processes that govern egg rejection require a certain constellation of cues at the same to operate efficiently, particularly if the hosts used a learned self-referencing technique, as Moskat et al. (2014b) suggests. The data demonstrates that older hosts are better rejecters than hosts naïve to their own egg appearance, but the authors suggest that other learned associations with parasitism may act as cues as well (such as sightings of a cuckoo female). Finally, all perceptual models should be rerun and analyzed again against the physical rejection rates documented here once the physiological characterization of the visual system of the great reed warbler is completed to determine whether the sensory system of this common and broadly studied common cuckoo host has itself shifted in unexplored ways during the evolutionary arms race against common cuckoos and their mimetic eggs.

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**Ethical approval:** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**References**


**Figure 3.1:** Four common cuckoo eggs and one host egg in the nest of a great reed warbler, representing a natural case of multiple parasitism in Hungary. The four cuckoo eggs differ in shape, color and speckling, so likely they were laid by different females (Moksnes et al. 2008). The single host egg is in the middle of the left side of the picture. (Photo credit: Csaba Moskát)
Figure 3.2: Treatment a: 1 egg in a clutch was painted blue, green, red, yellow, or orange, or replaced by a natural cuckoo or conspecific host egg (depending on the published field experiment from which the data were derived); treatment b: 3 eggs in a clutch were individually painted one color each (blue, yellow, and orange); treatment c: 3 eggs in a clutch were all painted the same single color (blue, yellow, or orange); treatment d: all eggs in a clutch were individually painted one color each (blue, green, red, yellow, and orange); treatment e: all eggs were painted the same single color (blue, yellow, or orange).
**Figure 3.3:** The relationship between rejection rates of artificial parasitic eggs and the chromatic perceptual difference (JND) from host eggs. Linear regression lines are plotted based on only the colors/egg types included and shown in each figure panel. The first chart displaying treatment a includes the cuckoo egg, the conspecific egg, and all five painted own egg colors, the second includes only the five painted own egg colors, and the third includes only blue, orange, and yellow colors which were the only colors used in treatments b, c, and e. The first chart displaying treatment d includes all five painted own egg colors, while the second only includes blue, orange, and yellow colors (which were the only colors used in treatment e).
Table 3.1: The relationship between rejection rates and chromatic perceptual differences (JND$_{50\%}$) in own-foreign egg appearances in each treatment. B = blue painted own egg, Y = yellow painted own egg, O = orange painted own egg, G = green painted own egg, Cu = natural common cuckoo egg, Ho = natural conspecific egg of another host individual. Bolded p-values indicate significance.

<table>
<thead>
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<th>Perceptual Differences</th>
<th>n (nests)</th>
<th>$R^2$</th>
<th>$p$</th>
<th>JND$_{50%}$</th>
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<tr>
<td>Treatment a (Cu, Ho, B, Y, O, G, or R)</td>
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<td>0.32</td>
<td>$&lt; 0.0001$</td>
<td>3.4</td>
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<tr>
<td>Treatment a (B, Y, O, G, or R)</td>
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<td><strong>0.0004</strong></td>
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<tr>
<td>Treatment a (B, Y, O)</td>
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<td>Treatment b</td>
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<td>0.41</td>
<td><strong>0.0007</strong></td>
<td>8.0</td>
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<tr>
<td>Treatment c</td>
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<td>0.14</td>
<td>0.0758</td>
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<tr>
<td>Treatment d (B, Y, O, G, R)</td>
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<td>Treatment d (B, Y, O)</td>
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<td>Treatment e</td>
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<td><strong>0.0053</strong></td>
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Chapter 4: Contrasting Perceptual Models of Egg Rejection along a Natural Color Gradient in a Host Species in Sympathy vs Allopatry from its Brood Parasite
Contrasting Perceptual Models of Egg Rejection along a Natural Color Gradient in a Host Species in Sympatry vs Allopatry from its Brood Parasite

Tommy Manna, Daniel Hanley, Peter Samas, Jarkko Rutila, Mikus Abolins-Abols, Marcel Honza, and Mark E. Hauber

Abstract

Hosts of avian brood parasites can mitigate the fitness cost of raising unrelated offspring by rejecting foreign eggs from the nest. A cognitive mechanism often thought to be used by a host to discriminate a foreign egg is to compare it against the hosts’ own egg(s) by color, maculation, shape, and/or size. However, Hanley et al. (2017) recently suggested that egg color variation along a continuous scale of natural avian eggshell coloration may instead be the salient recognition cue (i.e. directional chromatic contrast), and, in support, they found that using only the absolute difference between foreign vs. own eggs (absolute chromatic contrast) yielded an inferior statistical prediction for experimentally-induced egg rejection patterns. Here we conducted a parallel experiment where we compared absolute chromatic, directional chromatic, and absolute achromatic contrasts of painted immaculate artificial eggs as predictors for rejection patterns in Common Redstart *Phoenicurus phoenicurus*, often parasitized by a mimetic-egg laying Common Cuckoo *Cuculus canorus* host-race. Directional contrast was found to be the best predictor of rejection in both areas of
sympatry (Finland) and allopatry (Czech Republic) with the cuckoo, as our hosts rejected eggs noticeably browner but not noticeably bluer eggs than their own. These results support the paradigm of a single egg color rejection threshold and contribute to a growing generality of these patterns across diverse avian host-brood parasite systems.

**Key words:** brood parasitism, conspecific acceptance threshold, recognition systems.
Introduction

One of the common and best explored defense mechanisms employed by some hosts of avian brood parasitism is the visual discrimination and subsequent rejection of foreign eggs from the nest (Rothstein 1990, Lahti 2006, Soler 2015, Manna et al. 2017). Until recently, prevailing interpretations of the cognitive mechanism of foreign egg discrimination have dictated that the absolute degree of difference in color between the foreign egg(s) and the host’s own eggs is best predictor of rejection (Stoddard and Stevens 2011, Stevens, Troscianko, & Spottiswoode 2013, Hauber et al. 2015).

However, when Hanley et al. (2017) investigated and Hanley et al. (2019) followed up on three host species’ responses to artificial foreign eggs painted along a gradient of natural avian eggshell coloration (e.g. Hanley et al. 2015), they discovered and confirmed a novel cognitive decision rule: specifically, European blackbirds (Turdus merula) and American robins (T. migratorius), as well as Chalk-Browed Mockingbirds (Mimus saturninus) reject model eggs not only on the basis of absolute avian-perceivable color distance from the foreign and the hosts’ own eggs, but on the basis of the directional pattern of whether that difference was towards the shorter (bluer) or longer (browner) peak reflectance wavelengths (as judged by human perceivable colors). All three host species in those studies preferentially rejected eggs browner than their own but preferentially accepted similarly bluer/greener eggs. These findings implied that a single acceptance/rejection threshold exists at some distance from the host’s own egg color toward the browner end of
the scale, instead of the two symmetrical thresholds, equidistant from the host’s own egg color hypothesized by previous experimentation.

However, none of the focal host species in the studies by Hanley and colleagues were parasitized by brood parasites laying highly-mimetic eggs, implying that egg rejection patterns may have been due to generalized responses to any foreign eggs in the nest, rather than anti-parasitic adaptations against mimicry. Here we aimed to investigate the existence of single or multiple acceptance thresholds in the Common Redstart \((Phoenicurus phoenicurus;\) hereafter: redstart), a frequently parasitized host of the Common Cuckoo \((Cuculus canorus;\) hereafter: cuckoo) in a well-established host-parasite study system in Finland (Samaš, Rutila, & Grim 2016). We studied both this Finnish population and a more southern population of the redstart in the Czech Republic, the latter of which is not parasitized by the cuckoo currently (M. Honza and P. Samas, pers. comm.). Using 3D printed artificial eggs painted along a continuous blue/green to brown color gradient of natural avian eggshells (Hanley et al. 2015), we artificially parasitized redstart nests and observed whether rejection occurred. If the single threshold model developed by Hanley et al. applies to redstarts, then we expect host response to be dependent on the direction of color variation, and not just absolute difference from this species’ own egg coloration. Furthermore, if the frequency of interaction with the brood parasite shapes the evolution of rejection thresholds in this species (Reeve 1989), then we predict a narrow, more restrictive rejection threshold in Finland vs. the Czech Republic.
Methods

(a) Study Area and Experimental Procedures

We studied redstarts near Ruokolahti (61°24'N, 28°37'E) in south-eastern Finland and in the vicinity of Bzenec (48°56'N, 17°15'E) in south-eastern Czech Republic, from May to June, 2018. The Finnish population of redstarts is heavily parasitized by the cuckoo (Rutila, Latja, & Koskela 2002), while the Czech population is not (M. Honza and P. Samas, pers. comm.). We monitored redstart breeding attempts in nest boxes throughout the study areas, and placed one of our artificial eggs chosen randomly in the nest cup once the female had laid four eggs (typical clutch size is seven eggs in Finland; Rutila, Latja, & Koskela 2002). We proceeded to check on the content of each experimental nest daily for rejection, taking note of additional laying and natural cuckoo parasitism as it occurred. If the nest was still active and our artificial egg still in the nest cup after 6 days of checks, the host female was deemed an acceptor. If the foreign egg disappeared from the box or was found outside of the nest cup inside the box within that six day period, the host female was deemed a rejecter. We also used a ILM1335 RS Pro light meter (manufactured in Northamptonshire, UK) to obtain light level readings both in and outside the nest box on the onset of that nest box’s experimental progress to assess light conditions for viewing foreign eggs.

In all, 34 such experiments were completed in the Czech study area, and 50 were completed in the Finnish study area. In addition, 19 control nests were observed (9 in the
Czech Republic, 10 in Finland). These were procedurally identical in handling and monitoring the experimental clutches with regard to nest box manipulation and daily observation/notation, but no foreign egg model was introduced by the researchers.

(b) Artificial Eggs and Color Analysis

Eggs were printed with a MakerBot Replicator Mini+ (Brooklyn, USA) from a polylactic acid plastic filament. We painted these eggs colors with mixed paints ranging from blue/green to brown along a continuous spectrum of natural avian eggshell colors (for detailed methods, see Canniff et al. 2018). Each paint color was used on two eggs, and pairs of identically color eggs were split to ultimately make two identical sets of eggs to use in each of the two geographic study sites. The redstart lays an immaculate blue egg (Stoddard and Steven 2011) and we painted our artificial eggs without spotting. We then used reflectance spectrometry to quantify these colorations in the avian visible (300 nm-700 nm) range for each artificial egg, as well as 15 fresh redstart eggs in the field using a spectrometer (Jaz, Ocean Optics, Dunedin, Florida).

We then modeled the redstart visual system by calculating photoreceptor quantum catch based on the comprehensive visual sensory information available for the Blue Tit (*Cyanistes caeruleus*) photoreceptor sensitivities and relative cone/double cone densities (Hart et al. 2000, Hart 2001, Hanley et al. 2017, as the redstart and blue tit have either a close relative (e.g. European Blackbird) or themselves are, respectively, known to have a UV sensitive (UVS) color vision (Bowmaker et al. 1997). Further, the blue tit has been
used in previous work to model the redstart visual system successfully (Avilés 2008). We were thereby able to calculate perceived chromatic and achromatic distances between the average host egg and each of the model eggs in units of just noticeable difference (hereafter JND). A JND of 1 represents a threshold of predictably noticeable discriminability between the model egg and the host eggs, while a JND less of 1 deems the model egg color as visually indistinguishable. A JND higher than 1 indicates increased discernibility proportionally.

In order to better test our proposed single threshold framework, we calculated a third JND value by multiplying the chromatic JND (which provides absolute distance, regardless of direction on the blue/green to brown scale) of model eggs by either -1 or 1, depending on whether the egg color was more blue/green or more brown than the host species’ egg mean coloration along the gradient, respectively (sensu Hanley et al. 2017). In this way we could examine directional difference as well as absolute difference and compare their relative predictive strength (Canniff et al. 2018).

(c) Statistical Analyses

In order to determine which measure of JND best accounted for the most variance in patterns of egg rejection behavior, we ran generalized linear mixed models (GLMMs) in R version 3.5.2 (R Core Team 2013). We generated three GLMMs predicting host response by the directional JND, the chromatic JND, and the achromatic JND. Each of these models included locality (Finland vs Czech Republic), date, and light level as fixed effects. The
AIC (with AICc correction) was calculated for all models to assess and compare which cognitive model of eggshell coloration assessment best predicts experimentally-induced host responses.

Results

In all, the redstarts rejected the artificial egg in 23 out of 84 total nests (27.4%). The rejection rate in Finland (24%) and the Czech Republic (32%) did not differ statistically ($\chi^2 = 0.71$, $p = 0.553$). In turn, no rejection of eggs or nest desertion by the host female was observed in any of these control nests.

The directional contrast was the best predictor of rejection (model $R^2 = 0.104$, $p = 0.005$), though the absolute chromatic contrast was a poorer but still significant predictor (model $R^2 = 0.081$, $p = 0.012$). The directional contrast analysis allows us to determine that artificial eggs with colors of up to 2.5 JND more blue/green than the redstart eggs were much more likely to be accepted than 2.5 or more JND browner eggs (Figure 4.1). The absolute achromatic contrast was not a significant predictor of rejection. In all of the models, the fixed effects of geographic locality, date, and light level had no significant contributions (Table 4.1). The likelihood ratio tests indicated that the directional JND ($p = 0.016$) and chromatic JND ($p = 0.045$) were significantly better predictors than the null model. However, the AICc analysis selected the directional JND as model with the best fit and neither of the other two models or the null model were within $\Delta$AICc $\leq 2.0$ from this top model (Table 4.2).
Discussion

The redstart is an attractive experimental subject for studies of host-parasite interactions due to its acceptance of naturally laid mimetic cuckoo and mimetically painted artificial eggs and the rejection of non-mimetic painted natural or artificial eggs (Rutila, Latja, & Koskela 2002; Avilés, Rutila, & Møller 2005, Hauber et al. 2014, Samaš, Rutila, & Grim 2016). It has been suggested that, as redstarts are cavity nesters, cuckoo chicks are less successful in evicting all host chicks from the nest (Grim et al. 2009), and female cuckoos are prone to laying errors (e.g., missing the nest cup entirely when laying into the cavity; Samaš, Rutila, & Grim 2016). The resulting low-to-intermediate cost of cuckoo parasitism to the redstart allows for relaxed selection pressure for the rejection of foreign eggs and therefore is predicted to generate variable egg rejection behavior (Hauber et al. 2014). This allowed us to test in detail the perceptual-cognitive basis of redstart egg rejection behavior, and especially the extent of the need and type of mimicry required for accepting artificial eggs.

Here we demonstrated that variable egg rejection in the redstart extends beyond acceptance of mimetic and rejection of non-mimetic eggs, and instead we found that the directional contrasts of the artificial eggs were the best predictor for egg rejection, conforming with and confirming Hanley et al. (2017) and (2019) results and conclusions. Here the redstarts more often rejected browner eggs relative to their own species’ blue-green coloration. In fact, the only eggs with a negative directional JND (so, more blue/green than the host species’ eggs) that were rejected had a chromatic JND of <1,
indicating that the difference in color should not have been perceptible to the redstarts. The pattern (Figure 4.1) therefore establishes a single threshold of foreign egg rejection for this species, too. The probability of rejection reaches 50% at approximately 5.4 JNDs browner eggs along the directional JND analysis, potentially indicating this as an approximate threshold for rejection (Figure 4.1).

Contrary to predictions of the conspecific acceptance threshold hypothesis (Reeve 1989), Czech and Finnish cuckoos did not differ in their overall rejection rate or the position of their acceptance threshold. This may mean that variation in egg rejection behavior is maintained in this species regardless of the extent of actual parasitism pressure experienced by different populations; in support of this scenario are data from another Finnish redstart population where egg rejection of both mimetic and non-mimetic eggs is nearly absent, despite heavy patterns (but low effective) parasitism by cuckoos (Thomson et al. 2016).

Determining how a host decides to engage in anti-parasite behavior is critical to the study of any host-parasite system (Manna et al. 2017). For avian brood parasitism in particular, we do not have the luxury of being sensitive to many of the relevant visual cues ourselves (e.g., ultra-violet reflectance: Cherry & Bennett 2001), and so designing such investigations and conducting the resulting analysis require instrumentation and avian perceptual modeling (Aviles 2008, Cassey et al. 2008). The adoption of the single threshold model of rejection suggested by Hanley et al. 2017 and reinforced by Hanley et al. (2019) and now here should increase our predictive power with only a slight shift in analytical
methodology (Canniff et al. 2018), making it a tractable change of paradigm, and we advocate for it enthusiastically.

Acknowledgments: This work would not have been possible without the collaboration of Daniel Hanley of Long Island University, USA, Marcel Honza, Peter Samas, and others, of the Institute for Vertebrate Zoology, Czech Republic, and Jarkko Rutila, Finland. We thank Mikus Abolins-Abols for statistical assistance. For financial support I thank Mark Hauber’s Harley Jones Van Cleave Professorship at the University of Illinois at Urbana-Champaign.
References


**Figure 4.1:** Data points and logistical probability of egg rejection along the absolute chromatic (a), achromatic (b), and directional, blue/green to brown gradient (c) (in units of JNDs). Spot hues show human-visible colors of the painted artificial eggs.
Table 4.1: Model outputs of the GLMMs predicting egg rejection based on the three JND types tested as alternative predictors.

**Brown-blue JND**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2.82</td>
<td>0.005*</td>
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<td>Date</td>
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<td>0.83</td>
<td>0.410</td>
</tr>
<tr>
<td>Locality</td>
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<td>-1.16</td>
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<td>Light</td>
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<td>0.35</td>
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**Chromatic JND**

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<td>-1.56</td>
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<td>2.52</td>
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<td>Date</td>
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<td>0.31</td>
<td>0.71</td>
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<tr>
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<td>-1.07</td>
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<td>Light</td>
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**Achromatic JND**
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<td>Achromatic JND</td>
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<td>1.46</td>
<td>0.143</td>
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<tr>
<td>Date</td>
<td>0.12</td>
<td>0.30</td>
<td>0.39</td>
<td>0.700</td>
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<tr>
<td>Locality</td>
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<td>0.61</td>
<td>-0.95</td>
<td>0.341</td>
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<tr>
<td>Light</td>
<td>-0.15</td>
<td>0.31</td>
<td>-0.49</td>
<td>0.625</td>
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Table 4.2: Information theoretic scores of model AICc's and likelihood ratio tests against the null model; p < 0.05: *. The gray shaded model is the top model, with both of the other models outside the ΔAICc < 2.0 range.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>$\chi^2$ (df)</th>
<th>p</th>
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<tr>
<td>Brown-blue JND+ locality + date + light +1</td>
<td>99.12</td>
<td>10.26 (3)</td>
<td>0.016*</td>
</tr>
<tr>
<td>Chromatic JND + locality + date + light +1</td>
<td>101.35</td>
<td>8.03 (3)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Null + 1</td>
<td>102.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achromatic JND + locality + date + light +1</td>
<td>106.04</td>
<td>3.34 (3)</td>
<td>0.342</td>
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Chapter 5: Recognition, speciation, and conservation: recent progress in brood parasitism research among social insects

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Recognition, Speciation, and Conservation:
Recent Progress in Brood Parasitism

Research Among Social Insects

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Abstract

Obligate brood parasitism is costly to hosts because they are manipulated to invest in unrelated offspring. In insects, this has culminated in an evolutionary arms race of adaptations and counteradaptations between hosts and parasites, providing a unique mosaic of specialization and speciation to investigate arms races in the context of ecological dynamics. Recent progress has employed new techniques to challenge well-established notions such as nestmate recognition mechanisms in host species and revealing never before documented specialized adaptations of both parasites and their hosts. Newly constructed molecular phylogenies have allowed the opportunity to examine the relatedness of host-parasite species-pairs with unprecedented clarity, lending to discussions of social parasitism as a model of speciation in sympatry. Finally, the recent and destructive spread of a lethally brood parasitic subspecies of honey bee in South Africa is discussed.
Introduction

A brood parasite is an organism which manipulates others to raise its offspring, forgoing the cost of parental investment. Naturally, then, brood parasitism tends to evolve in taxa heavily associated with intensive postnatal parental care for offspring; such as birds, fishes, and social insects. The phenomenon is perhaps most extensively studied in birds, where a fantastic coevolutionary story has been unfolding as parasite trickery and host discrimination race to keep up with one another[1]. In all cases, the fitness cost of rearing non-kin offspring[2] inflicts a selective pressure on parental hosts to counter adapt, often resulting in compelling evolutionary narratives of an arms race between pairs of host and parasite taxa.

Social insects in particular represent an attractive study system in this context. Their relatively straightforward life histories and precise behavioral repertoires allow for uncomplicated interpretation of results from unambiguous experimental manipulations. Brood parasites in the insect world are commonly referred to instead as "social parasites" as they are viewed as exploiting a large scale collaborative eusocial network of individuals and reaping gains without any relevant contribution[3]. The most common strategy employed by parasitic bees[4], wasps[5], and ants[6] is that of an inquiline invader; queens of these species, which have usually lost their worker caste evolutionarily, sneak into unrelated host colonies and either usurp or coexist with the host queen, chemically manipulating the host workers to raise their own parasitic brood. Even some species of butterfly have been demonstrated to employ this strategy, their larval forms residing in the nests of ants and mimicking queen-specific cues to receive nourishment from the host
workers[7,8]. More drastic strategies also exist, such as that of several species of "slave-making" ants, whose worker caste has none of the standard custodial ability and instead is specialized in conducting "slave raids" on neighboring unrelated nests of host ant species and returning to their own colonies with the uneclosed brood of the host species in tow. Once the enslaved workers emerge, they are forced to carry out the basic duties of a standard working caste of ants for the parasitic slave-maker species' colony[9,10*].

In this brief review, the past few years of discovery in host-parasite dynamics within social insects is examined, including complete revamping of longstanding ideas of nestmate recognition, the state of innovative counteradaptations including "slave rebellion" behavior in host ant species, the most recent discussions on the opportunity of social parasitism to provide examples of speciation in sympatry, and the case of a newly prolific subspecies of parasitic honeybee which threatens an entire region's ecosystem.

**Nestmate Recognition and Deception**

In the case of birds, the evolved ability to visually identify one's own eggs is a powerful tool mediating parasitic foreign egg rejection in avian brood parasites’ hosts. In turn, nestmate recognition systems in insects are a critical line of defense against social parasites attempting to integrate into the colony and therefore represent an active angle of investigation in this field[11–15]. Signorotti et al. published a transformative study in 2014 challenging long-held notions about the ontogeny of a *Polistes* paper wasp commonly targeted by social parasites obtaining its nestmate odor template from nest-paper in a critical period immediately following eclosion. The authors exposed newly eclosed *P.*
dominulus workers to either paper from their own nest, paper from a foreign conspecific nest, or a control neutral condition. Surprisingly, all three exhibited proficient nestmate recognition capabilities when returned to their home nests, suggesting that nest material sensed immediately following eclosion of an adult worker is not, as was assumed, the means by which a P. dominulus worker obtains its nestmate recognition template. This calls into question many standing notions regarding the avenue by which the many social parasites of P. dominulus in its native range hijack the familial recognition system of the nest they are invading[14].

While host insect species must evolve means to recognize foreign invaders within their colonies and nests, social parasites must in turn develop strategies not to be recognized. Advances in molecular, behavioral, and morphological techniques now allow for a more thorough examination of chemical deception employed by these invaders than ever before[16–19*,20]. Accordingly, Nehring et al. in 2015 examined the stages of social and chemical integration of the inquiline Acromyrmex insinuator parasitic ant into the nest of its host, the leaf cutting ant Acromyrmex echinatior. While A. echinatior workers are shown to lethally attack foreign conspecific queens, the authors demonstrate that heterospecific parasitic A. insinuator queens are accepted by the host workers after an initial bout of sublethal aggression. The authors are able to present the invading queen's cuticular hydrocarbon signal progressively across these stages, showing that initial nest entry and reduction of aggression are likely due to a chemical signature that indicates insignificance as features normally essential to identify an individual are absent.
Subsequently, the parasite undergoes a camouflaging process and comes to mimic the colony-specific odor of its host, resulting in total acceptance[17].

**Current Adaptations in the Coevolutionary Arms Race**

As the adaptive landscape of a parasite-host arms race is always shifting, there are opportunities for never-before seen and captivating new coadaptations to discover. The past few years of brood parasitic social insect research have been no exception both in regards to parasite adaptations to their hosts[18,19*,21,22] and vice versa[23,24*–26]. Sulger et al. presented a fascinating finding in their 2014 study on reduced brain tissue investment in an obligate slave-making ant species relative to their host. Postulating that since social parasite *Polyergus mexicanus* delegates most cognitive problem solving to the enslaved workers of its host species *Formica fusca* (which are solely responsible for foraging, brood rearing, and nest excavation), it may exhibit reduced levels of energetically costly neural tissues. Indeed, the authors confirmed lower relative investment in mushroom bodies of the brain commonly associated with complex tasking in social insects[21]. This parallels similar findings of reduced brain sizes in parasitic cuckoo taxa relative to non-parasitic cuckoo lineages[27].

One of the more exciting developments in host defense in recent years is the discovery of "slave rebellion" behavior in worker ants enslaved by slave-makers[23,24*,28,29,30*]. First described in detail by Achenbach et al. in 2009, enslaved individuals of *Temnothorax longispinosus* were observed to cull many of the parasite pupae in their charge. Indeed, in some regions up to 77% of parasitic *Protomognathus*
Americanus pupae were determined to have been killed by their *T. longispinosus* caretakers. It was previously speculated that since enslaved workers never reproduce, a post-parasitism defense mechanic could not affect individual fitness and therefore could not emerge. However, it is now accepted that this particular behavior likely acts as kin-selected defense to protect neighboring, related, and still non-parasitized colonies of *T. longispinosus* by drastically reducing subsequent *P. americanus* generations' raiding power[31,32].

Another convincing post-parasitism host adaptation is described in Cini et al.'s 2014 observation of parasitized *Polistes* wasp workers' enlarged ovaries in relation to control conspecifics. It is part of the parasitic *Polistes sulcifer* queen's behavioral repertoire to suppress the reproductive activity of the host species workers in her usurped nest. However, the authors demonstrate that parasitized *P. dominulus* workers invest in ovary development and begin to more opportunistically lay their own eggs in response to the presence of the social parasite[26].

**Speciation and Emery's Rule**

Broadly speaking, Emery's rule in brood parasite-relevant entomology states that a social parasite tends to target a species or subspecies to which it is closely related[33,34]. Two interpretations of the principle have emerged over many years: the loose and the strict form. The loose form is often linked to allopatric evolution of the parasite-host dynamic in which a parasite will adapt to a closely related host species (often occupying the same or a neighboring phylogenetic clade) since shared physiology or social heuristics can allow for ease of integration into the host nest[34]. The strict form, however, states that a social
parasite sympatrically speciates directly from an immediate common ancestor of its host, representing a true pair of sister species[33,34,36–38*,39].

In recent years, phylogenetic studies based on genetic information have produced evidence of social parasite phenotypes emerging from both processes. For example, Rabeling et al. published a molecular phylogeny analysis in 2014 showcasing powerful evidence that the inquiline parasitic ant *Mycocepurus castrator* evolved reproductive isolation from its fungus growing host *Mycocepurus goeldii* in sympatry, adhering to the strict version of Emery's rule[40]. Additionally, Leppanen et al. described in 2015 what the authors interpret as an ongoing speciation between two morphs, one intraspecifically parasitic, of the ant *Myrmicarubra*. While their analyses demonstrated that the two morphs are not yet reproductively isolated, the authors describe the spatial isolation between breeding populations of the wild morphs and how restricted gene flow is allowing speciation to proceed[41].

While sympatric emergence and the strict form of Emery's rule seem to be gaining traction in the last few years in ant parasites[42], recent findings in the other members of the order hymenoptera come out on the opposite side. Smith et al. conducted a molecular phylogeny comparison in 2013 on allodapine bees, demonstrating that while most host-parasite pairs are closely related and adhere at least to the weak, allopatric model of social parasite evolution, none are true sister species one would expect to find under the strict sympatric model[43]. Similarly, Lopez-Osorio et al. published a phylogenetic analysis based on 12 gene fragments of *Dolichovespula* and *Vespula* yellowjackets and *Vespa* hornets finding that they, too, did not evolve sympatrically from their hosts and support
only the weak version of Emery's rule[36]. Finally, Messer et al. reported in 2016 their discovery of a new ant social parasite species (the first of its genus): *Nylanderia deceptrix*. The authors state that research to place *N. deceptrix* phylogenetically in relation to its obligate host, *Nylanderia parvula*, has still not been carried out, and so to which competing Emery’s rule evolutionary scenario *N. deceptrix* will lend its support is yet to be discovered[44].

**Thelytoky and the Cape Honey Bee**

Thelytoky is a mode of asexual reproduction in which a gametic polar body fuses with the egg pronucleus following meiosis, resulting in a diploid egg which develops normally into a pseudo-clone of the maternal individual. While extremely rare in vertebrates, thelytoky is most commonly observed in arthropods and is disproportionately prevalent in the cape honey bee *Apis mellifera capensis*, a South African subspecies of the western honey bee[45,46]. Individuals of this subspecies exhibiting this trait are also equipped for social parasitism, and will invade the nests of unrelated colonies of different honeybee subspecies, and begin to lay their brood, which are cared for and raised by the native host workers. These offspring are usually reproductives also capable of thelytoky, and contribute nothing to the upkeep of the host colony while advancing their life cycles and laying their own brood until the nest population's proportion between contributing native workers and parasitic loafers becomes unsustainable and results colony death. At this time, the remaining generation of parasitic *capensis* disperses and each individual can potentially invade and infect a colony of her own, lending to the recent virulent spread of
this subspecies and consequential epidemic honeybee colony collapse across Africa[39,47].

The devastating effects of this trend were documented in a 2014 report by Pirk et al. in which managed bee keepers of South Africa report a total colony loss of 29.6% in the 2009-2010 season and 46.2% in the 2010-2011 season, identifying the parasitic *capensis* as the primary cause[48]. The allelic underpinning of thelytoky in *capensis* were thought to be understood[46] but a study carried out by Chapman et al. in 2015 determined through a reciprocal backcrossing design that the appearance of thelytoky is inconsistent with a model that implicates a single locus. Furthermore, the authors reveal the presence of the 9bp deletion originally speculated to trigger thelytoky in subspecies of *A. mellifera* both in and outside of Africa that do not exhibit thelytoky[49]. Possibly exasperating the problem, Holmes et al. published a 2014 discovery of asexually produced queen cells in a colony of *Apis cerana*, the first observed instance of thelytokous reproduction in a honey bee other than *A. mellifera*. The authors expound on the possible role of this discovery in recent invasions by populations of that species[50]. Social parasitism facilitated by thelytoky in honey bees is a particularly poorly understood immediate danger to biodiversity and ecological dynamics reliant on bee populations, and is in desperate need of collaborative scientific problem solving.

**Conclusion**

It is an exciting era in the study of brood parasitic social insects; from complete reworking of basic ideas of species recognition and mechanisms of nest infiltration to new
interpretations of phylogenies made possible by methodological advances which spark spirited discussion regarding modalities of speciation. Future work is required not only to build on these exciting novel foundations but to obtain a handle on such poorly understood mechanisms as the inheritance of thelytoky in the brood parasitic honeybee subspecies, *Apis mellifera capensis*. Furthermore, despite the many parallels with the well-studied avian brood parasitism literature, there are several important areas of insect social parasite research on which very little empirical data yet exists. For instance, just as many parasitic bird chicks come equipped with postnatal mechanisms to further manipulate their host parental unit to not only accept them but treat them preferentially[2], many insect social parasite larvae have been demonstrated not only to avoid policing by host workers but to receive disproportionate attention[51,52], the mechanisms of which are yet to be convincingly detailed. Future work combining molecular, ontogenetic, and behavioral mechanisms of host manipulation and acceptance by parasitic insects will provide a productive new integrative research frontier.

**Conflict of Interest**

None to declare.

**Funding**

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workers as queens in the Eastern honeybee Apiserana. [Internet]. *Heredity (Edinb).* 2014, 114:1–4.


Prospectus

Hosts of avian brood parasites’ parental instincts are being fully exploited from the egg state to fledging by increasingly sophisticated parasitic mimicry and supernormal stimuli (Soler et al. 2003, Dawkins & Krebs 1979). In turn, these hosts are evolving detection and recognition behaviors with growing scrutiny in one of the most compelling cases of a co-evolutionary arms race available for focal and comparative studies (Spottiswoode & Stevens 2012). My collection of articles and manuscripts focuses primarily on host defense at the egg stage, where hosts with varying levels of discriminatory proficiency are tasked with identifying and removing foreign eggs of varying levels of mimicry.

Chapter 1 represents a comprehensive review of the collective progress in modelling the cognitive rulesets that govern a host’s decision to reject foreign eggs. It was Rothstein (1975) who first posited and drew the distinction between the two candidate rulesets: rejecting the “odd egg out” in the nest (discordancy) or rejecting eggs perceived as distinct from a known internal template of the host’s own eggs. The discordancy explanation was accepted as the sole factor at play for a long time, in part because of its parsimony and in part because on the surface it predicted natural and experimentally induced egg rejection patterns. After all, a parasitic egg is very likely to be in the minority of a clutch’s appearance of the host’s own eggs (Hauber 2001). However, incidental variation in a host’s own clutch may cause an overly sensitive rejecter to reject its own eggs under discordancy. This strategy is especially vulnerable in the context of multiple
parasitism (also called repeated targeting), where the hosts’ own eggs may come to represent the minority in a clutch (Stevens et al. 2013). In fact, over time as efforts were made to tease apart these mechanisms with focused experimental testing, evidence began to mount convincingly in favor of the template mechanism (e.g., Lotem et al. 1995, Lahti & Lahti 2002, Lang et al. 2014).

Chapter 1 proceeds then to review a series of experiments including those done by Moskát et al. which demonstrate that discordancy and template based discrimination may be happening in tandem. Moskát et al. (2010) demonstrated that the great reed warbler *Acrocephalus arundinaceus*, a host species of the mimetic common cuckoo *Cuculus canorus*, exhibits recognition processes with elements of both discordancy and template-based mechanisms, even when artificially parasitized nests were designed to activate only one mechanism. These same researchers then in 2014 demonstrated how the discordancy mechanism’s interaction with the template mechanism of this host was mimicry-context dependent, and as multiple foreign eggs in the nest varied in heterogeneity only certain minority colors were rejected. This potential disruptive interaction between competing mechanisms was followed up in Chapter 3 of this dissertation.

Chapter 2 is a large scale experimental effort and analysis in response to conflicting reports on the propensity for the house sparrow *Passer domesticus* to reject foreign eggs. Three reports from one study group published very high rejection responses (between 24% and 46%) in house sparrows to experimental parasitism in South Africa (López de Hierro and Ryan 2008) and in Granada, Spain (Lopez de Hierro and Moreno-Rueda 2010, Soler et
al. 2011). However, a similar study on a sparrow population in China by a different research group yielded nearly no rejection behavior above baseline (Yang et al. 2015). Since the house sparrow is a ubiquitous invasive species across many biomes and ecosystems, it represents an attractive potential global model for egg rejection, should it prove to be a rejecter. To examine this ourselves, we rigorously carried out similar experiments on populations in North America, Israel, and New Zealand. In all three geographic regions egg rejection rates were negligible and not statistically different from background rates of the disappearance of control eggs, suggesting that the house sparrow is not a suitable model species for foreign-egg rejection experiments on a global scale.

Chapter 3 returns to the subject of cognitive mechanisms of rejection, this time examining the question posed earlier in Chapter 1: what happens when simultaneous cognitive mechanisms interfere with one another, such as when they produce different targets for rejection? We focused on great reed warblers experimentally parasitized with painted (highlighter-marked) own eggs. We used reflectance spectrometry to estimate perceptual distances from each experimental egg to the host species unmanipulated eggs. We then incorporated existing published datasets (Bártol et al. 2002, Hauber et al. 2015, Bán et al. 2013) and analyzed new data under varying treatment conditions of clutch context to assay whether an interaction between rejection mechanisms results in reduced rejection efficiency.

We found that, in cases where the hosts’ own eggs were in the minority of the clutch and the foreign eggs were of uniform color, hosts exhibited differential response
rates to differently mimetic foreign egg colors, suggesting the employment of a template based mechanism (as a purely discordancy based heuristic should preferentially reject the minority regardless of appearance). We also confirmed (e.g. Ban et al. 2013, Stevens et al. 2013) that discrimination thresholds became more permissive as the proportion of foreign eggs in the nest was increased, and as the variability in appearance of those foreign eggs was made greater. This indicates that a discordancy based mechanism is also activated and interferes with the template based mechanism in cases of multiple parasitism, ultimately leading us to conclude that multiple parasitism itself can compromise this host’s defenses (sensu Stevens et al. 2013). Indeed, our host population has been subjected to consistently and unusually high levels of multiple parasitism over the last century (Zölei et al. 2015). However, due to the nestmate lethality of *Cuculus canorus* chick eviction behavior, the overall fitness benefit of such a strategy in this system is theoretically very limited (Takasu & Moskát 2011). We conclude Chapter 3 by suggesting the need for repeating of this analysis once complete information on the great reed warbler’s visual system is made available; to more precisely calculate perceptual distances between egg colors in this system.

In Chapter 4 we followed up recent work by Hanley et al. (2017 and 2019) and conducted a parallel study to identify whether and where a directional rejection threshold in a brood parasitic host may exist. Traditionally, absolute chromatic contrast (total distance of colors between host and foreign egg, regardless of direction) has been accepted as the most salient visual cue for rejection decisions (Hauber et al. 2015). However, when Hanley et al. subjected three separate host species to artificial parasitism using eggs painted on a
continuous blue/green to brown gradient of all natural avian eggshell colors, they
discovered that the direction of that distance was the most important factor, as hosts
preferentially rejected eggs browner than their own, but not more blue/green than their
own.

We therefore set out to collect further evidence for this phenomenon using the
common redstart *Phoenicurus phoenicurus*, a host species under parasitic pressure from the
mimetic-egg laying *Cuculus canorus* (Rutila et al. 2002). The common redstart, as opposed
to the house sparrow discussed in Chapter 2, is a highly suitable model for egg rejection
behavior due to its acceptance of highly mimetic eggs and rejection of non-mimetic eggs,
as the limits of mimicry and thresholds for rejection can be tested directly (Samaš et al.
2016).

We utilized 3D printing of artificial eggs and painted them along a continuous color
gradient of natural avian eggshell background colors (Canniff et al. 2018). Using the
reflectance spectrometry of these and natural host eggs, as well as avian visual modeling,
we calculated the avian-perceived distance between these eggs and host eggs in units of just
noticeable difference (JND). We also quantified the absolute chromatic contrasts as well as
achromatic contrasts, and we enumerated directional contrast by assigning the chromatic
contrasts of eggs more blue/green than that of the host a negative number, so the host’s
eggshell color would represent a 0 on a sliding scale that can go in each direction (Hanley
et al. 2017).
We then artificially parasitized active redstart nestboxes at two study sites, one in Finland where the redstarts were simultaneously under heavy parasitic pressure from cuckoos (~50%), and one in the Czech Republic where they were not (0%). As hypothesized, we found directional contrasts to be the best statistical predictors of rejection relative to eggshell color, as our hosts overwhelmingly rejected browner eggs vs. more blue/green eggs. This result comes in support of an important but tractable shift in perspective on the both modeling and the characterization of rejection thresholds in hosts of avian brood parasitism, and we hope that future work in the visual modeling of host behavior will follow suit of this general methodology (Caniff et al. 2018).

Finally, in Chapter 5, we shift perspective on host-parasite interactions by switching between lineages entirely, as we review studies examining brood parasitism in social insects. Brood parasitism is often instead called “social parasitism” in the context of insects, as they are viewed to be exploiting a large scale collaborative eusocial network of host colonies and reaping gains without any relevant contribution to colony productivity. Just as in avian brood parasitism, offspring rearing individuals are “tricked” with sensory cues to raise non-kin, parasitic offspring, and a resulting arms race of nestmate recognition and parasitic mimicry has followed (Lenoir et al. 2001, Cervo 2006, Buschinger 2009).

From inquiline strategies such as those employed by parasitic paper wasps wherein parasitic species’ queens invade host nests, either killing or coexisting with the host queen, and remain unidentified as an intruder through chemical trickery, to more drastic strategies such as the “slave maker” variants of parasite ants which invade host nests en masse to
capture uneclosed workers, returning them to the parasite nest where they are made to serve the custodial needs of the parasites, which do not have a worker caste of their own (Blatrix & Herbers, 2003), a wide variety of parasitic attack and host defensive strategies have evolved.

However, full understanding of these insect host-parasite systems is far from complete. Specific parasitic mechanisms, such as parasite larvae receiving disproportionate attention from host caretakers, compared to host larvae, are poorly characterized, even when the analogous avian mechanism is well established and studied (Cervo et al. 2004 vs. Kilner et al. 2005). Subsequent studies that have already cited this article have also called for wider adoption of insects as models for brood parasitism to broaden our perspective and make use of the system-specific experimental advantages (Cini et al. 2019, Smith & Belk 2018).

In all, this collection of articles and manuscripts serves to better characterize decision-based host defense behaviors against brood parasites, and several of the cognitive models we use to approximate them. My hope is that future studies will shed further light on the interactions between separate cognitive mechanisms, attend to directional contrasts as the most salient visual cue to influence rejection, and set out to characterize the visual systems of effective experimental host models, such as the great reed warbler and the common redstart, to improve the accuracy of the perceptual models we use for these studies. Finally, I conclude by extending my thanks to the Graduate Center of the City University of New York, my dissertation committee, and Dr. Mark Hauber.
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