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Reject the odd egg: egg recognition mechanisms in parrotbills

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Studies of the behavior of animals when confronted with tasks differing in complexity can improve our understanding of animal cognition and learning mechanisms. Coevolutionary interactions between brood parasites and their hosts provide an ideal opportunity for studying animal cognition because egg recognition and rejection are some of the most important adaptations evolved in hosts to counter brood parasitism. The cognitive mechanisms hosts employ in egg recognition have received substantial interest, with 2 main hypotheses being put forward: 1) true egg recognition based on a knowledge of the hosts’ own egg appearance (template that is innate and/or learned) and 2) discordancy by which individuals simply recognize eggs that are in minority as parasitic. These hypotheses are not necessarily mutually exclusive. We conducted egg experiments in the ashy-throated parrotbill (Paradoxornis alphonsianus), a common cuckoo (Cuculus canorus) host that lays immaculate, polymorphic eggs, and we obtained support for both hypotheses. Parrotbills use the presence of own eggs as a cue for recognizing parasitic eggs, supporting true recognition, but without the presence of own eggs as a template they failed to recognize the parasitic egg. Furthermore, some individuals erroneously rejected their own eggs when in minority, supporting recognition by discordancy. Such a combination of cognitive mechanisms that involves true recognition and discordancy in egg recognition in a single population has as far as we can tell never previously been described.

Key words: brood parasitism, cognition, cuckoo, discordancy, egg recognition, online processing.

INTRODUCTION

Decision making depends on a number of related cognitive processes, and studies of animal cognition can improve our understanding of this process (Hauber and Sherman 2001). Avian brood parasites like the common cuckoo (Cuculus canorus, hereafter cuckoo) lay eggs in host nests and thus transfer the cost of parental care to their hosts (Davies and Brooke 1989). Hence, successful parasitism conversely triggers the evolution of anti-parasite behavior like rejection of the parasitic egg (Davies and Brooke 1989; Moksnes et al. 1991). Interactions between brood parasites and their hosts provide an ideal opportunity for studying animal cognition because egg rejection is based on cognitive mechanisms related to recognition of the parasitic egg (Rothstein 1974; Hauber and Sherman 2001). Two major hypotheses have been developed to explain cognitive mechanisms responsible for egg recognition in brood parasite hosts: 1) true or template-based recognition, in which hosts imprint on their own egg color (innate ability and/or by learning) as a template for rejecting alien eggs and 2) recognition by discordancy, by which hosts reject eggs that are present in minority (no innate template or learning required) (Rensch 1925; Rothstein 1974, 1975; Hauber and Sherman 2001; Bán et al. 2013; Stevens et al. 2013). Most studies so far have obtained support for true or template-based recognition with or without learning, a combination of these, and various duration of the sensitive period for learning (Rothstein 1974, 1975; Moksnes 1992; Lotem et al. 1995; Scaly and Bazin 1995; Lahti and Lahti 2002; Lyon 2007). Recognition by discordancy has received far less support (Rensch 1925; Rothstein 1975; Lyon 2007). Recently, recognition based on true recognition and discordancy has been shown to work in tandem as both effects of recognition and discordancy have been found in the same study (Marchetti 2000; Mokáň et al. 2009, 2010; Bán et al. 2013; Stevens et al. 2013). These latter studies have all in common that the host species in question lay maculate eggs. Hence, it was not...
straightforward in these studies to disentangle the importance of ground color and spotting pattern on cognitive output and rejection decisions. 

Ashy-throated parrotbills (Paradoxornis alphonsianus, hereafter parrotbills) are cuckoo hosts with polymorphic egg colors that constitute defenses against cuckoo parasitism. There is evidence of disruptive selection on egg color in both cuckoos and parrotbills (Yang et al. 2010), and theoretical models have shown that the maintenance of egg polymorphism only occur if hosts have a high sensitivity of rejecting parasitic eggs (i.e., good capabilities of rejection) (Liang, Yang, Stokke, et al. 2012). Parrotbill eggs are either immaculate blue, pale blue or white, with each female laying eggs of one specific type. Interestingly, the cuckoo also lays polymorphic eggs of the same colors to match those of the host clutches, but there are no indications of active selection of nests with the “correct” host egg color in the parrotbill cuckoo genus (Yang et al. 2010, 2013). In a previous study, it was shown that parrotbills possess an acute ability to recognize and reject parasitic eggs (Yang et al. 2010), but the underlying mechanisms remain unknown. Here, we tested parrotbill cognitive abilities by experimentally parasitizing nests using real, conspecific eggs. If discordancy was at work, we would predict an effect of the number of eggs deviating in color from the original color of eggs of the nest owner being in minority, whereas true recognition in our experiment was predicted to result in rejection of alien eggs deviating from the hosts’ own egg type.

MATERIALS AND METHODS

This study was performed in Kuankuoshui National Nature Reserve (KKS), Guizhou, Southwestern China, where 11 species of cuckoos breed sympatrically. Parrotbills are regularly parasitized by common cuckoos in the area (Yang et al. 2010). We systematically searched for parrotbill nests in April–August 2009–2013. Parrotbill mean clutch size was 4.52 eggs (standard deviation = 0.65, n = 175), and we assigned nests to 12 treatment groups (Figure 1) by manipulating the clutch combinations as either 1 (a1–a2), 2 (b1–b2), 3 (c1–c2), or 4 (d1–d2) of 4 host eggs exchanged with 1 parasitic egg to obtain a clutch size of 4 eggs. Responses were classified as 1) parasitic eggs rejected, 2) own eggs rejected, or 3) all eggs accepted. Rejection included both desertion and ejection because there was a statistically significant difference in desertion rate between experimental and control groups (12/109 vs. 0/40, respectively, \( \chi^2 = 4.79, \text{ degrees of freedom } [df] = 1, P = 0.03 \)), confirming that desertion was a specific response against the parasitic egg. For responses 1) and 2), all parasitic eggs or own eggs were ejected, respectively, whereas for desertion, all eggs in the clutch were left in the nest. We investigated the relationship between behavior and treatment using a logistic regression vs. b2, c1 vs. c2, d1 vs. d2, e1 vs. e2, chi-square tests, all \( P > 0.61 \). Therefore, experimental groups that received the same treatment were merged into 6 groups for further statistical analyses. The logistic regression model of rejection behavior fitted the data (Table 1; goodness-of-fit statistic, \( \chi^2 = 191.84, df = 246, P = 1.00 \)). There was a significant effect of treatment (Figure 2 and Table 1).

RESULTS

Rejection responses to blue and white host clutches with the same treatments were not significantly different (Figure 2; a1 vs. a2, b1 vs. b2, c1 vs. c2, d1 vs. d2, e1 vs. e2, chi-square tests, all \( P > 0.61 \)). More specifically, although the effect of the number of eggs that differed in color from the original one was significant, the effect of color of original eggs and the interaction between color of original eggs and number of eggs that differed from the original one were not significant. There was a higher rejection rate later in the season (Table 1). None of the experimental nests were parasitized by cuckoos.

DISCUSSION

We disentangled cognitive mechanisms used in parasitic egg recognition by a brood parasite host laying immaculate, polymorphic colored eggs. Three major conclusions could be drawn from our experiments. Firstly, hosts laying blue and white eggs consistently exhibited the same responses to all treatments. Secondly, parrotbills showed an acute ability to reject parasitic eggs even when their own eggs were in minority similar to several other species (e.g., Victoria 1972; Rothstein 1975; Moksnes 1992; Sealy and Bazin 1995; Lyon 2007). However, when no host eggs were present as a template, most pairs accepted the parasitic eggs. These results support a cognitive mechanism described as online self-referencing (Hauber and Sherman 2001; Bán et al. 2013). Rensch (1925) similarly observed acceptance of foreign painted eggs in a few experiments, and a Phylloscopus species accepted full sets of host eggs that were swapped with oversized eggs, although such eggs were rejected if one of the original host eggs were returned to the clutch (Marchetti 2000). However, other studies found that hosts were able to recognize foreign eggs even without any self-referencing (Victoria 1972; Rothstein 1975). A possible explanation for the apparent reliance of parrotbills on own eggs as a template for egg rejection may be related to the lack of spotting pattern on parasitic eggs used in this study. Hence, the cognitive task of distinguishing between blue and white eggs may have been excessively complicated without any template. Thirdly, when host eggs were outnumbered by parasitic eggs, 5 pairs ejected their own single egg, whereas the remaining pairs correctly ejected all 3 parasitic eggs. Ejection of own eggs never followed any other treatment. The erroneous ejection of the single host egg when in...
minority indicates that some host pairs base their recognition on discordancy rather than true recognition. Such variation in cognitive mechanisms used for egg recognition has also been found in other host species (Moskát et al. 2010; Bán et al. 2013; Stevens et al. 2013).

Parrotbill hosts use the presence of own eggs as a cue for recognizing parasitic eggs, supporting true recognition, but without the presence of own eggs as a template, they failed to recognize the parasitic egg. Furthermore, some individuals erroneously rejected their own eggs when in minority, supporting recognition by discordancy. Such a combination of cognitive mechanisms that involves true recognition and discordancy in egg recognition in a single population has never previously been described. Our study, therefore, constitutes an important part of the puzzle resolving cognitive mechanisms used in egg recognition by hosts of brood parasites.

What are the consequences of template-based recognition mechanism in parrotbills? Moskát et al. (2009) and Stevens et al. (2013) found that probability of egg acceptance increased with multiple parasitism. Unlike their host–parasite systems, where multiple parasitism is common, more than 1 cuckoo egg has never been found in our parrotbill nests. Hence, the egg recognition mechanisms in parrotbills are well suited to deal with cuckoo parasitism in natural situations. The different results obtained from various study species suggest that no single egg recognition mechanism is universal, but rather that there is variation among species. In parrotbills, we do not know the importance of learning for egg recognition. According to theoretical models, species like parrotbills with a low intrACLutch variation in egg appearance should not need a prolonged learning period, but rather imprint on their first egg or just have an internal template without any need for learning at all (Stokke et al. 2007). In parrotbills, both sexes are known to incubate and reject parasitic eggs. Hence, we predicted that the cognitive mechanisms used in egg rejection should be based on discordancy rather than learning because males may erroneously
reject own eggs if they had learned another egg color in a previous breeding attempt (Liang, Yang, Antonov, et al. 2012). These predictions should be tested in future studies.

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**Table 1**

Logistic regression model of the relationship between rejection behavior and original egg color, number of eggs that differed from the original one and their interaction and laying date

<table>
<thead>
<tr>
<th>Variable</th>
<th>χ²</th>
<th>df</th>
<th>P</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color of original eggs (C)</td>
<td>1.61</td>
<td>2</td>
<td>0.45</td>
<td>0.223</td>
<td>0.181</td>
</tr>
<tr>
<td>No. eggs that differed from original (N)</td>
<td>15.71</td>
<td>2</td>
<td>0.0004</td>
<td>0.400</td>
<td>0.129</td>
</tr>
<tr>
<td>C × N</td>
<td>1.33</td>
<td>2</td>
<td>0.51</td>
<td>−0.147</td>
<td>0.128</td>
</tr>
<tr>
<td>Laying date</td>
<td>11.61</td>
<td>2</td>
<td>0.003</td>
<td>0.065</td>
<td>0.024</td>
</tr>
</tbody>
</table>

The overall model had the statistics (χ² = 26.61, df = 8, P = 0.0008).

Figure 2

Percentage of rejection and acceptance by parrotbills in the 10 experimental groups. Numbers in brackets above the bars refer to the sample sizes and below the abscissa, ratios of host eggs versus alien eggs, respectively.
Sealy SG, Bazin RC. 1995. Low frequency of observed cowbird parasitism on eastern kingbirds: host rejection, effective nest defense, or parasite avoidance? Behav Ecol. 6:140–145.
