OXFORD
UNIVERSITY PRESS

INSTRUCTIONS

1. **Author groups:** Please check that all names have been spelled correctly and appear in the correct order. Please also check that all initials are present. Please check that the author surnames (family name) have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors' full surnames and forenames are tagged correctly, for accurate indexing online. Please also check all author affiliations.

2. **Figures:** If applicable figures have been placed as close as possible to their first citation. Please check that they are complete and that the correct figure legend is present. Figures in the proof are low resolution versions that will be replaced with high resolution versions when the journal is printed.

3. **Colour reproduction:** These figures are currently intended to appear online in colour and black and white in print. Please check the black and white versions (these may be available at the end of the paper) and contact us if you have any concerns. Please re-word the legend/text to avoid using reference to colour. Alternatively, please let us know if you wish to pay for print colour reproduction or to have both versions in black and white. Please note that there is a £350/$600 charge for each figure reproduced in colour in print.

4. **URLs:** Please check that all web addresses cited in the text, footnotes and reference list are up-to-date, and please provide a 'last accessed' date for each URL.

5. **Funding:** Please provide a Funding statement, detailing any funding received. Remember that any funding used while completing this work should be highlighted in a separate Funding section. Please ensure that you use the full official name of the funding body, and if your paper has received funding from any institution, such as NIH, please inform us of the grant number to go into the funding section. We use the institution names to tag NIH-funded articles so they are deposited at PMC. If we already have this information, we will have tagged it and it will appear as coloured text in the funding paragraph. Please check the information is correct. [red text to be used for suppliers who are tagging the funding]

6. **Conflict of interest:** All authors must make a formal statement indicating any potential conflict of interest that might constitute an embarrassment to any of the authors if it were not to be declared and were to emerge after publication. Such conflicts might include, but are not limited to, shareholding in or receipt of a grant or consultancy fee from a company whose product features in the submitted manuscript or which manufactures a competing product. The following statement has been added to your proof: 'Conflict of Interest: none declared'. If this is incorrect please supply the necessary text to identify the conflict of interest.

7. Please note that the first colour figure of this paper will be published in print colour at no charge.

MAKING CORRECTIONS TO YOUR PROOF

These instructions show you how to mark changes or add notes to the document using the Adobe Acrobat Professional version 7.0 (or onwards) or Adobe Reader 8 (or onwards). To check what version you are using go to **Help** then **About**. The latest version of Adobe Reader is available for free from get.adobe.com/reader.

For additional help please use the **Help** function or, if you have Adobe Acrobat Professional 7.0 (or onwards), go to http://www.adobe.com/education/pdf/acrobat_curriculum7/acrobat7_lesson04.pdf
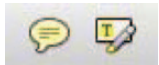
Displaying the toolbars

Adobe Reader 8: Select Tools, Comments & Markup, Show Comments and Markup Toolbar**. If this option is not available, please let me know so that I can enable it for you.**

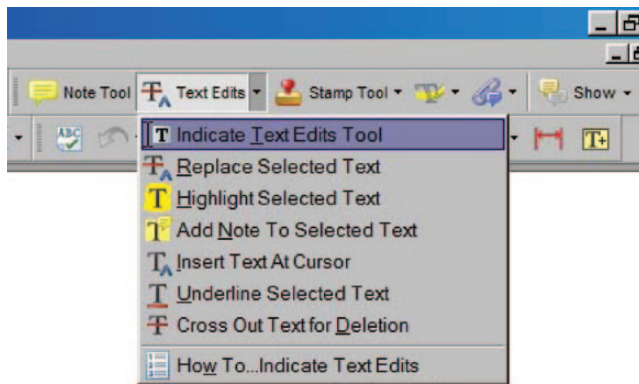**Acrobat Professional 7: Select** Tools, Commenting, Show Commenting Toolbar**.**
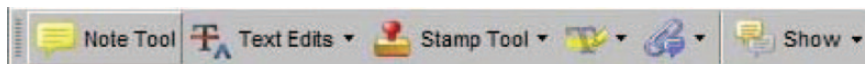
Adobe Reader 10: To edit the galley proofs, use the Comment Toolbar (Sticky Note and Highlight Text).

Using Text Edits

This is the quickest, simplest and easiest method both to make corrections, and for your corrections to be transferred and checked.

1. Click **Text Edits**

2. Select the text to be annotated or place your cursor at the insertion point.

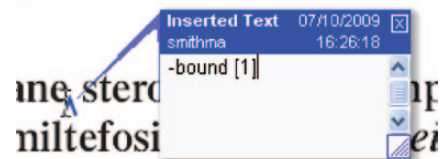3. Click the **Text Edits** drop down arrow and select the required action.

   *You can also right click on selected text for a range of commenting options.*

Pop up Notes

With *Text Edits* and other markup, it is possible to add notes. In some cases (e.g. inserting or replacing text), a pop-up note is displayed automatically.

To **display** the pop-up note for other markup, right click on the annotation on the document and selecting **Open Pop-Up Note**.

To **move** a note, click and drag on the title area.
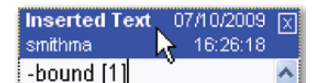
To **resize** of the note, click and drag on the bottom right corner.

To **close** the note, click on the cross in the top right hand corner.

To **delete** an edit, right click on it and select **Delete**. The edit and associated note will be removed.

SAVING COMMENTS

In order to save your comments and notes, you need to save the file (**File, Save**) when you close the document.
A full list of the comments and edits you have made can be viewed by clicking on the Comments tab in the bottom-left-hand corner of the PDF.

AUTHOR QUERY FORM

Journal	:	BEHECO
Article Doi	:	10.1093/beheco/aru124
Article Title	:	Reject the odd egg: egg recognition mechanisms in parrotbills
First Author	:	Canchao Yang
Corr. Author	:	Wei Liang

AUTHOR QUERIES - TO BE ANSWERED BY THE CORRESPONDING AUTHOR

The following queries have arisen during the typesetting of your manuscript. Please answer these queries by marking the required corrections at the appropriate point in the text. Failure to do so could result in delayed publication.

AQ1	Please check that all names have been spelled correctly and appear in the correct order. Please also check that all initials are present. Please check that the author surnames (family name) have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors' full surnames and fore-names are tagged correctly, for accurate indexing online. Please also check all author affiliations.
AQ2	Please provide the name of full road and district address (if any) for affiliations a–c.
AQ3	Figures have been placed as close as possible to their first citation. Please check that they have no missing sections and that the correct figure legend is present.
AQ4	Please check the change made to the sentence "We investigated the relationship…"
AQ5	Figure 2 is in low resolution, please provide us the high resolution figure for processing.

Behavioral Ecology

Original Article

Reject the odd egg: egg recognition mechanisms in parrotbills

Canchao Yang,[a] Anders Pape Møller,[b] Eivin Røskaft,[c] Arne Moksnes,[c] Wei Liang,[a] and Bård G. Stokke[c]

[a]Ministry of Education Key Laboratory for Tropical Plant and Animal Ecology, College of Life Sciences, Hainan Normal University, Haikou 571158, China, [b]Laboratoire Ecologie, Systematique et Evolution, UMR 8079 CNRS-Université Paris-Sud XI-AgroParisTech, Batiment 362, F-91405 Orsay Cedex, France, and [c]Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway

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 antiparasite 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; Sealy 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; Moskát 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

Address correspondence to W. Liang. E-mail: liangwei@hainnu.edu.cn.

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 gens (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 dissimilarly colored egg; all 4 host eggs exchanged with similarly colored eggs (e1–e2); or nests were visited and monitored in the same way as experimental nests but without manipulation (f1–f2, $N = 20$, not shown in Figure 1). True recognition allows the host to reject alien eggs no matter the number of own eggs present in the nest, whereas recognition by discordance favors rejection of the egg type in minority. Hence, groups a1 and a2 were assigned to test the egg recognition ability of hosts when alien eggs were in minority; groups b1–b2 and c1–c2 allowed us to investigate the influence of the 2 recognition mechanism when the cognitive tasks were more complicated; groups d1 and d2 were assigned to investigate the egg recognition by hosts when the template (its own egg color) was removed; groups e1–e2 and f1–f2 were used to control for the effect of egg color and human disturbance on host nests, respectively. Combining these groups, we can investigate the effects of egg color on the cognitive ability and mechanisms of egg rejection by parrotbills.

All experiments were conducted within 1–2 days after clutch completion, and nests were monitored the following 6 days. In nests with 3 or 5 eggs, clutch size was increased by adding an egg of similar color or decreased by 1 egg, respectively, 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, $\chi^2 = 4.79$, 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 while controlling statistically for the effects of original egg color, the number of eggs that differed from the original color, and the interaction between original egg color and the number of eggs that differed from the original color and laying date as predictors.

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 $P > 0.61$). 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). 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 the 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

3.5

3.10

3.15

3.20

3.25

3.30

3.35

3.40

Group a1. blue clutch (3b + 1w)

Group a2. white clutch (3w + 1b)

Group b1. blue clutch (2b + 2w)

Group b2. white clutch (2w + 2b)

Group c1. blue clutch (1b + 3w)

Group c2. white clutch (1w + 3b)

Group d1. blue clutch (0b + 4w)

Group d2. white clutch (0w + 4b)

Group e1. blue clutch (0b + 4b*)

Group e2. white clutch (0w + 4w*)

3.65

3.70

3.75

3.80

3.85

3.90

3.95

3.100
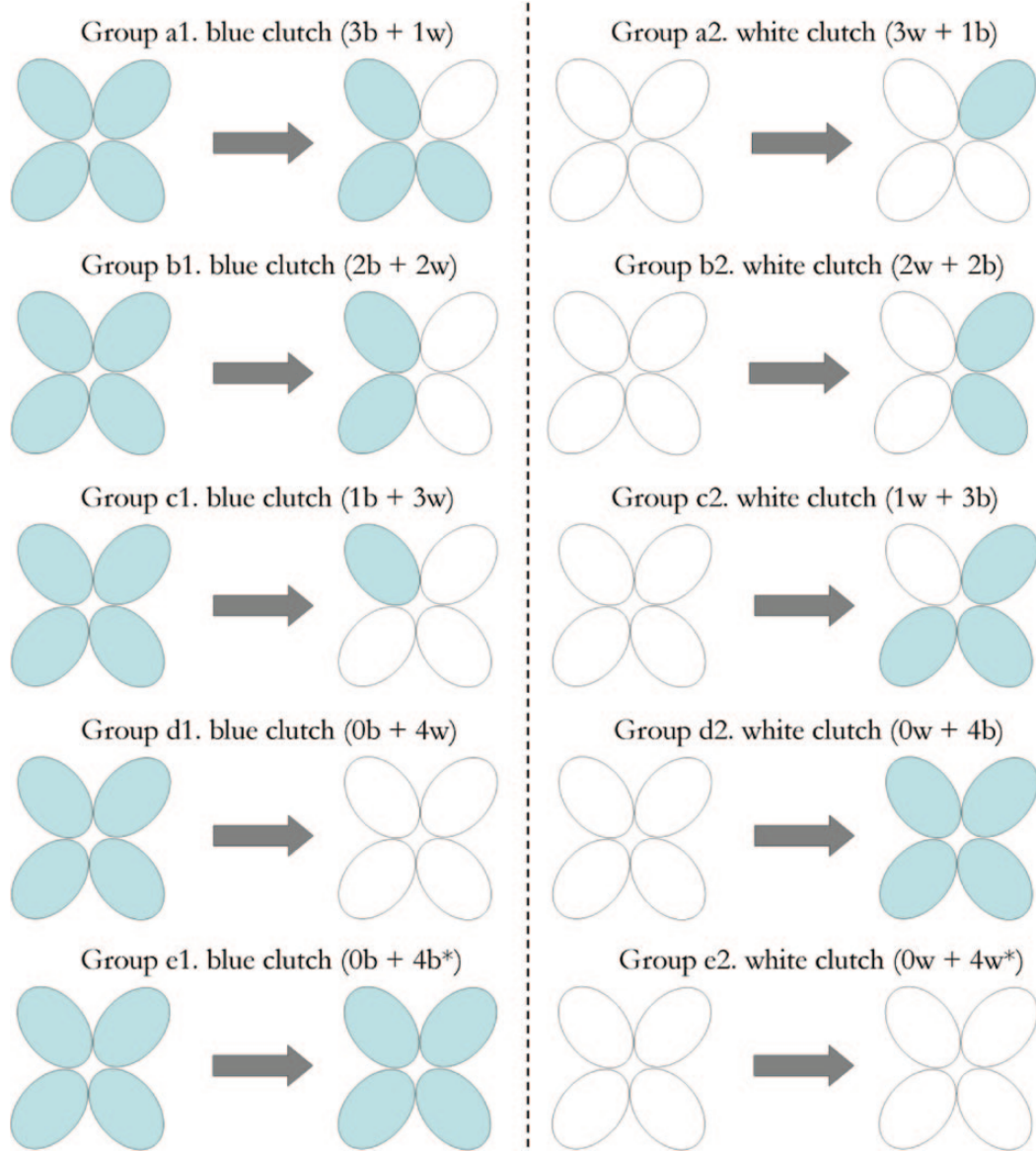
Figure 1
Treatments in the 10 experimental groups with b referring to blue eggs and w to white eggs within brackets. *Full set of conspecific eggs with similar color as host eggs.

AQ3
3.105

3.45 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).

3.50 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.

3.60 What are the consequences of template-based recognition
3.61 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
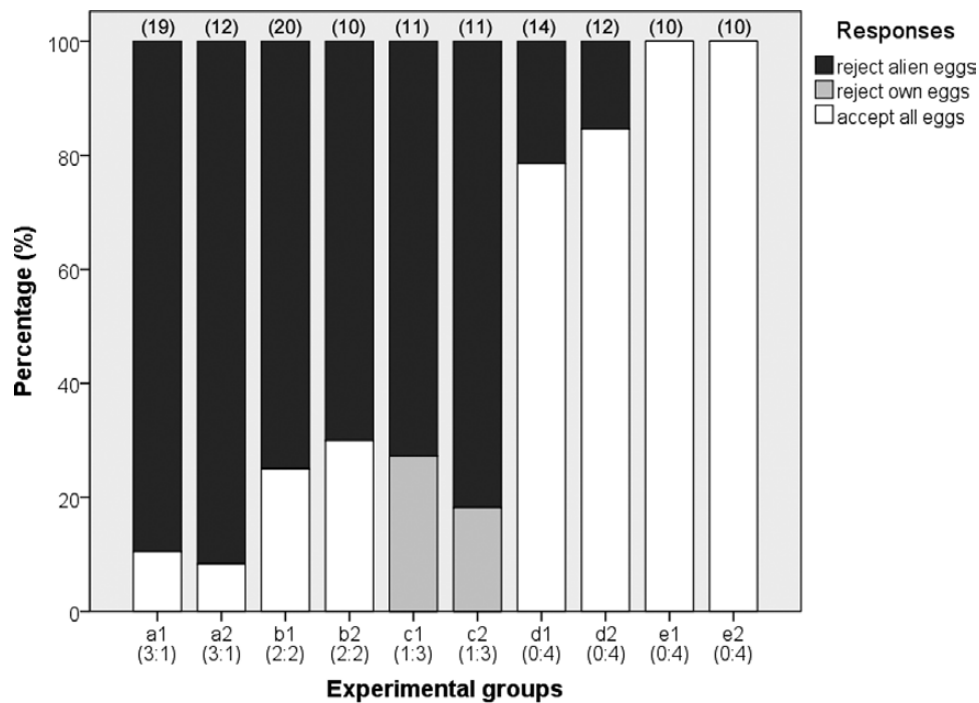
3.110

3.115

3.120

3.122

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.

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

Variable	χ^2	df	P	Estimate	SE
Color of original eggs (C)	1.61	2	0.45	0.223	0.181
No. eggs that differed from original (N)	15.71	2	0.0004	0.400	0.129
C × N	1.33	2	0.51	−0.147	0.128
Laying date	11.61	2	0.003	0.065	0.024

The overall model had the statistics ($\chi^2 = 26.61$, df = 8, $P = 0.0008$).

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.

Handling editor: Johanna Mappes

REFERENCES

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

Davies NB, Brooke MdeL. 1989. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. J Anim Ecol. 58:207–224.

Hauber ME, Sherman PW. 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. Trends Neurosci. 24:609–616.

Lahti DC, Lahti AR. 2002. How precise is egg discrimination in weaverbirds? Anim Behav. 63:1135–1142.

Liang W, Yang C, Antonov A, Fossøy F, Stokke BG, Moksnes A, Røskaft E, Shykoff JA, Møller AP, Takasu F. 2012. Sex roles in egg recognition and egg polymorphism in avian brood parasitism. Behav Ecol. 23:397–402.

Liang W, Yang C, Stokke BG, Antonov A, Fossøy F, Vikan JR, Moksnes A, Røskaft E, Shykoff JA, Møller AP, et al. 2012. Modelling the maintenance of egg polymorphism in avian brood parasites and their hosts. J Evol Biol. 25:916–929.

Lotem A, Nakamura H, Zahavi A. 1995. Constraints on egg discrimination and cuckoo-host co-evolution. Anim Behav. 49:1185–1209.

Lyon B. 2007. Mechanisms of egg recognition in defenses against conspecific brood parasitism: American coots (*Fulicaamericana*) know their own eggs. Behav Ecol Sociobiol. 61:455–463.

Marchetti K. 2000. Egg rejection in a passerine bird: size does matter. Anim Behav. 59:877–883.

Moksnes A. 1992. Egg recognition in chaffinches and bramblings. Anim Behav. 44:993–995.

Moksnes A, Røskaft E, Braa AT, Korsnes L, Lampe HM, Pedersen HC. 1991. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. Behaviour. 116:64–89.

Moskát C, Bán M, Székely T, Komdeur J, Lucassen RWG, van Boheemen LA, Hauber ME. 2010. Discordancy or template-based recognition? Dissecting the cognitive basis of the rejection of foreign eggs in hosts of avian brood parasites. J Exp Biol. 213:1976–1983.

Moskát C, Hauber ME, Avilés JM, Bán M, Hargitai R, Honza M. 2009. Increased host tolerance of multiple cuckoo eggs leads to higher fledging success of the brood parasite. Anim Behav. 77:1281–1290.

Rensch B. 1925. Verhalten von SingvögelnbeiÄnderung des Geleges. Ornithol Monatsber. 33:169–173.

Rothstein SI. 1974. Mechanisms of avian egg recognition: possible learned and innate factors. Auk. 91:796–807.

Rothstein SI. 1975. Mechanisms of avian egg-recognition: do birds know their own eggs? Anim Behav. 23:268–278.

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.

Stevens M, Troscianko J, Spottiswoode CN. 2013. Repeated targeting of the same hosts by a brood parasite compromises host egg rejection. Nat Commun. 4:2475.

Stokke BG, Takasu F, Moksnes A, Røskaft E. 2007. The importance of clutch characteristics and learning for antiparasite adaptations in hosts of avian brood parasites. Evolution. 61:2212–2228.

Victoria JK. 1972. Clutch characteristics and egg discrimination ability of the African village weaverbird *Ploceuscucullatus*. Ibis. 114:367–376.

Yang C, Cai Y, Liang W. 2013. Eggs mimicry of common cuckoo (*Cuculus canorus*) utilizing ashy-throated parrotbill (*Paradoxornis alphonsianus*) host. Chin Birds. 4:51–56.

Yang C, Liang W, Cai Y, Shi S, Takasu F, Møller AP, Antonov A, Fossøy F, Moksnes A, Røskaft E, et al. 2010. Coevolution in action: disruptive selection on egg colour in an avian brood parasite and its host. PLoS One. 5:e10816.