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Egg shape mimicry in parasitic cuckoos

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COMPETING INTERESTS

The authors declare no competing or financial interests.

AUTHOR CONTRIBUTIONS

M.R.G.A., I.M. and N.E.L. conceived the idea. E.S. designed the data collection procedure, and M.R.G.A. and I.M. collected the data. I.M. and E.S. analysed data and prepared figures. I.M. and M.R.G.A. wrote the manuscript, and all authors provided edits. *All authors gave final approval for publication.*

ABSTRACT

Parasitic cuckoos lay their eggs in nests of host species. Rejection of cuckoo eggs by hosts has led to the evolution of egg mimicry by cuckoos, whereby their eggs mimic the colour and pattern of their host eggs to avoid egg recognition and rejection. There is also evidence of mimicry in egg size in some cuckoo-host systems, but currently it is unknown whether cuckoos can also mimic the egg shape of their hosts. In this study we test whether there is evidence of mimicry in egg form (shape and size) in three species of Australian cuckoos: the fan-tailed cuckoo *Cacomantis flabelliformis* which exploits dome nesting hosts, the brush cuckoo *Cacomantis variolosus,* which exploits both dome and cup nesting hosts, and the pallid cuckoo *Cuculus pallidus,* which exploits cup nesting hosts. We found evidence of size mimicry, and for the first time evidence of egg shape mimicry in two Australian cuckoo species (pallid cuckoo and brush cuckoo). Moreover, cuckoo-host similarity was higher for hosts with open nests than for hosts with closed nests. This finding fits well with theory, since it has been suggested that hosts with closed nests have more difficulty recognising parasitic eggs than open nests, have lower rejection rates, and thus exert lower selection for mimicry in cuckoos. This is the first evidence of mimicry in egg shape in a cuckoo-host system,

suggesting that mimicry at different levels (size, shape, colour pattern) is evolving in concert. We also confirm the existence of egg size mimicry in cuckoo-host systems.

Keywords: egg mimicry; parasitism; cuckoo; shape; size; Fourier analysis

INTRODUCTION

Interspecific avian brood parasites lay their eggs in nests of other bird species, transferring parental care to their host (Davies, 2000). Host parents typically lose reproductive success and incur the extra cost of rearing unrelated offspring (Davies, 2000). Many host species have evolved defence mechanisms to escape or reduce the cost of parasitism, including rejection of parasite eggs and nestlings (Davies, 2000; Langmore *et al.*, 2003). Several host species recognise the appearance of their eggs and reject foreign eggs based on distinctions in egg size, colour and pattern (Langmore *et al.*, 2003; Stoddard & Stevens, 2010). In turn, this has led to the evolution of parasitic eggs that more closely mimic host clutches to reduce the risk of egg rejection (Spottiswoode & Stevens, 2012).

Egg shape is the least-studied component of egg mimicry though it has received more attention (but no evidence) in recent years (Bán *et al.*, 2011). Bird species can recognise and eject objects from their nests based on their shape (Underwood & Sealy, 2006; Zölei *et al.*, 2012). Egg form (shape and size) (Dryden & Mardia, 1998) could be part of the suit of traits (such as colour and pattern) that have evolved to match host egg phenotype and could evolve in concert with other traits.

We test whether there is evidence of mimicry in egg shape and size in Australian cuckoos. We focus on three generalist cuckoo species in Australia, the fan-tailed cuckoo *Cacomantis flabelliformis,* brush cuckoo *Cacomantis variolosus* and pallid cuckoo *Cuculus pallidus*. Each of these species exploits different host species (details in supplementary material), depending on the geographic region, which allows us to test our hypotheses using multiple host-cuckoo pairs. Crucially, two of these cuckoos (the brush cuckoo and the pallid cuckoo) exhibit host specific races, termed gentes. Thus, these two cuckoo species lay different egg types that match the colour and pattern of their host eggs (Beruldsen, 2003; Starling *et al.*, 2006). Given that there is variation in the nest type of their hosts, we also tested whether the accuracy of egg-form mimicry between cuckoo and host varies with nest type. It has been shown that the structure of the nest can have important implications in the evolution of mimicry (see discussion), because visual cues, such as colour and pattern, are less evident in low light conditions. The fan-tailed cuckoo exploits hosts with closed nests, the brush cuckoo exploits hosts with both types of nests, and the pallid cuckoo prefers to exploit hosts with open nests.

Previous studies of host egg size and shape mimicry by brood parasites have typically used measurements of egg length and width to calculate egg volume or the ratio of length to width (e.g., Mason & Rothstein, 1986, Spottiswoode *et al.*, 2011, but see Bán *et al.*, 2011). This approach neglects some potentially informative cues that are available to hosts, because egg shape can vary from spherical to elliptical and in degree of asymmetry between the blunt pole (round end) and sharp pole (pointed end) (Zölei *et al.*, 2012). Here we use elliptical Fourier descriptor analysis to capture variation in egg shape more accurately by focussing on the contours and geometry. Using this method, we provide the first evidence of mimicry in egg shape in the cuckoo-host system. Importantly, we show that the occurrence of egg shape mimicry is dependent on nest type.

MATERIALS AND METHODS

We photographed parasitised clutches of three cuckoo species and 16 host species (n= 511 eggs, Table S1, details in supplementary material). All clutches contained 1 cuckoo egg and 2 to 3 host eggs. Clutches were obtained from the Australian National Wildlife Collection, CSIRO, Canberra, and the Australian Museum, Sydney. To characterise egg shape we obtained two-dimensional outlines (x, y coordinates) from the photographs using Image J (version 1.48) as detailed in the supplementary material. Outlines of each egg were aligned with their long axis along the x-axis and subjected to an elliptical Fourier descriptor analysis (Kuhl & Giardina, 1982) resulting in 32 harmonics that approximated the egg outline, using the package *Momocs* (Bonhomme *et al.*, 2013) in the R interactive statistical environment (version 0.99) (R Core Team, 2016). Harmonics are elliptic descriptions of a closed contour, where the harmonics sequentially provide finer-scale approximations for the trends in the original periodic function (Kuhl & Giardina, 1982). A principal components (PC) analysis was performed on elliptical Fourier coefficients for each egg, and the first 10 PCs, accounting for 99% of the variation in shape, were retained for subsequent analyses (see Fig. S1 for biplot of PC1 and PC2). Egg length (the difference between the minimum and maximum coordinates in the x-axis, i.e., distance between the sharp and blunt pole) was used as a measure of egg size in this study.

Similarity in egg form between cuckoo and their host was measured using Euclidean distances representing the difference in size or shape between the cuckoo egg and each of its host's eggs within the same clutch; we refer to these as 'real distances'. Smaller distances indicate greater similarity in the specified egg characteristic. To explore whether there is evidence supporting mimicry in shape or size between cuckoo and host eggs we used a permutation procedure where the real distances between cuckoo-host pairs were compared to

a distribution of pairwise distances between cuckoo and randomly allocated non-host eggs (i.e., cuckoo eggs paired with host eggs from other cuckoo species); we refer to these as 'random distances'. A total of 6547 possible pairwise comparisons were used to compare real distances and random distances. To test whether 'real distances' in egg shape or size were smaller than the 'random distances' we used a linear mixed model, implemented in the package *lme4* (Bates *et al.*, 2015) and a generalized mixed model, as described below. We performed the following tests per cuckoo species and per host nest type, because these two factors are highly correlated and could not be used in the same model (e.g., all hosts of the pallid cuckoo have open nests and all hosts of the fan-tailed cuckoo have closed nests).

To test whether the degree of cuckoo-host egg similarity differed between 1) cuckoo species and 2) between hosts with closed and open nests, we built two linear mixed models for each response variable. The response variables were the distance in shape and size between each cuckoo egg and their hosts' eggs. Cuckoo species was the fixed predictor variable for one linear mixed model, and nest type of the host was the predictor variable for the other. In all models we included host species and clutch ID as random effects and response variables were transformed to increase normality and/or facilitate model convergence (log-transformed shape for a Gaussian distribution and square-root of size for a Gamma distribution).

RESULTS

Similarity in egg shape between cuckoo and real host species was significantly higher than comparisons between cuckoo and randomly allocated non-host eggs for hosts with open nests ($\chi^2 =17.12$, $P<0.001$), but not for closed nests ($\chi^2=0.27$, $P=0.60$; Table 1, Fig. 1A). Shape similarity between cuckoo and real host eggs was high for the pallid cuckoo (which exploits open nesters) and for some hosts of the brush cuckoo (those with open nests, GLMM, pallid

cuckoo $\chi^2 = 20.96$, $P<0.001$, brush cuckoo $\chi^2 = 5.61$, $P=0.02$). Host nest type had a stronger effect on cuckoo-egg shape similarity than cuckoo species (standardized estimate $\beta_{nest\ type}=0.31$ vs. $\beta_{cuckoo\ sp}=0.10$). Cuckoos that exploit hosts with open nests had eggs that resemble the shape of their hosts more closely (Table 1, Fig. 1B). Brush cuckoos, which exploit both open and closed nests, showed greater egg shape mimicry when they parasitise open nesting hosts (GLMM, $\chi^2 = 4.99$, $P=0.03$, Fig. 1B).

Differences in egg size between cuckoo and real hosts for both nest types (and all three cuckoo species) were significantly smaller than between cuckoo and non-host eggs (Table 1, Fig. 2A). Nest type or cuckoo species were not significant predictors of egg size similarity (Table 1, Fig. 2B). Egg outlines showing the largest differences in shape and size between cuckoo and host eggs are presented in Fig. S2. Fan-tailed cuckoo eggs were significantly larger than the eggs of their host species (ANOVA, $F_{4,35}=7.14$, $P<0.001$), whereas pallid cuckoo and brush cuckoo egg size was not significantly different to their host eggs (ANOVA, $F_{3,26}=0.732$, $P=0.54$ and $F_{6,8}=2.053$, $P=0.17$, respectively).

There were significant differences in cuckoo egg shape across nests of host species for the fan-tailed cuckoo ($F_{4,35}=4.015$, $P=0.009$), the pallid cuckoo ($F_{3,25}=3.397$, $P=0.003$), and the brush cuckoo ($F_{4,35}=2.58$, $P=0.032$, supplementary figure S3). There were also significant differences in cuckoo egg size across nests of host species for the fan-tailed cuckoo ($F_{4,35}=7.144$, $P<0.001$) and the brush cuckoo ($F_{6,8}=2.650$, $P<0.029$) but not for the pallid cuckoo ($F_{3,25}=0.732$, $P=0.542$, supplementary figure S3).

DISCUSSION

We provide the first evidence in support of egg shape mimicry among cuckoos and their hosts, and support previous experiments showing that hosts can discriminate and reject foreign eggs based on shape (Underwood & Sealy, 2006; Zölei *et al.*, 2012). Our findings further confirm that egg size mimicry can occur in cuckoos that exploit hosts with both open and closed nests (Krüger & Davies, 2004; Antonov *et al.*, 2010). Size is a very important cue for egg rejection (Marchetti, 2000). Small egg size has evolved in many cuckoo genera possibly as an adaptation to parasitise smaller hosts (Krüger & Davies, 2004).

Eggs of the pallid cuckoo and the brush cuckoo were more similar in shape and size to eggs of their own hosts than to eggs of hosts of other cuckoo species. This finding agrees with our expectations, since the pallid and the brush cuckoo have been reported to show different gentes, or egg phenotypes, that mimic in colour and pattern the eggs of their hosts (Beruldsen, 2003; Starling *et al.*, 2006). Therefore, our findings suggest that there is multi-component egg mimicry in these cuckoos, and that colour, pattern, size and shape mimicry might be evolving in concert in these cuckoo eggs. However, within the brush cuckoo we found that shape egg similarity was high for hosts with open nests, but low for hosts with closed nests. This suggests that shape similarity between cuckoo and host eggs can be influenced not only by the cuckoo species, but also by differences in nest type of the host. In fact, cuckoo-host egg shape similarity could be explained better by differences in host nest type than by differences in cuckoo species – although the strong link between cuckoo species and host nest type makes it difficult to disentangle the effect of both variables. The only previous study on egg shape mimicry could not find evidence of egg shape similarity in different gentes of the common cuckoo (*Cuculus canorus*) (Bán *et al.*, 2011). However, a different method was used (e.g. landmarks) and the sample size for hosts was smaller.

It has been previously suggested that closed nests may constrain the evolution of egg mimicry because there is no selection from egg rejection (Langmore *et al.*, 2005). Open nests are built with the interior directly exposed to the sun (e.g., cup or platform) while closed nests other than cavities are built with a covering, creating an enclosed chamber (e.g., dome, pendant or spherical nest) (Møller, 1989), with lower visibility. In fact, hosts with open nests present higher rates of rejection than closed nests (Langmore *et al.*, 2005). Moreover, colour and pattern mimicry is suggested to be better in brood parasites that exploit hosts with open nests (Davies, 2000; Langmore *et al.*, 2009, although see Igic *et al.*, 2012). Poor visibility in closed nests may limit the host's ability to recognise foreign eggs, and has been used to explain the lack of egg rejection (and cuckoo egg mimicry) in some species (Brooker & Brooker, 1989; Langmore *et al.*, 2005). However, rather than relying on visual cues, some hosts with closed nests could rely on tactile cues to reject foreign eggs of the wrong size (Mason & Rothstein, 1986; Langmore *et al.*, 2003). This is supported by our results, where similarity in egg size was stronger for real distances than random distances, regardless of species and nest type. This suggests that hosts may use visual and tactile senses to distinguish between eggs based on their size. Likewise, when multiple female brood parasites exploit the same nest they may preferentially remove the eggs of other brood parasites, rather than host eggs (Spottiswoode, 2013; Gloag *et al.*, 2014), and this too can select for mimicry of host egg size and shape in brood parasites (Spottiswoode *et al.*, 2011).

Our results provide evidence of higher cuckoo-host egg shape similarity than expected by chance in two species of Australian cuckoos and we suggest that egg shape mimicry could be the mechanism behind this pattern. Moreover, within the same cuckoo species (brush cuckoo), which exploits both open and closed nesting hosts, there is a higher degree of similarity in egg shape when eggs were laid in open nests. In conclusion, our study suggests

that selection for some components of mimicry (such as egg shape) may vary not only across cuckoo species, but also between nest types, whereas others (such as egg size) are important identifying features for both nest types.

DATA AVAILABILITY

Data will be submitted to Dryad Digital Repository upon acceptance.

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Table 1. Results of linear mixed models comparing differences in size and shape between cuckoo and host eggs. Significant results are shown in bold. Type of comparison refers to whether the distances were calculated between cuckoo and host eggs within the same clutch (real distances) or between cuckoo and randomly allocated non-host eggs (random distances).

	Type of comparison	$\chi 2$	*P*-value	Estimate β
Shape	Real distances in open nests vs. Random distances	17.12	**< 0.0001**	-0.138
	Real distances in closed nests vs. Random distances	0.27	0.601	-0.018
	Distances in open nests vs. Distances in closed nests	13.09	**0.0002**	-0.317
Size	Real distances in open nests vs. Random distances	189.93	**< 0.0001**	-0.761
	Real distances in closed nests vs. Random distances	7.54	**0.006**	-0.152
	Distances in open nests vs. Distances in closed nests	0.031	0.859	0.031

FIGURE LEGENDS

Figure 1. Distribution of differences in egg shape (A) between cuckoo-host pairs and (B) between cuckoo and host eggs laid in closed nests and open nests. In panel (A), differences between cuckoo eggs and real host eggs are shown in green and red, and differences between cuckoo eggs and randomly allocated non-host eggs are shown in blue. In panel (B), points denote average per host species with standard error bars.

Figure 2. Differences in egg size (A) between cuckoo-host pairs and (B) between cuckoo and host eggs laid in closed nests and open nests. See Figure 1 for details. The outlier in panel (B) (blue bar, closed nest) corresponds to *Acanthiza pusilla*, a host of *Cacomantis flabelliformis* that has much smaller eggs than its parasite.