Clutch variation and egg rejection in three hosts of the pallid cuckoo, *Cuculus pallidus*

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Summary

In theory, hosts of avian brood parasites would benefit by modifying their egg appearance in two ways to help identify mimetic foreign eggs: (i) by laying clutches that are more uniform in appearance and (ii) by laying clutches that differ from those of other females in the population. Support for these theories is inconsistent, and few studies have used objective measures of clutch variation. Here we used reflectance spectrophotometry to quantify within-clutch and between-clutch variation of three host species of an Australian brood parasite, the pallid cuckoo (*Cuculus pallidus*). We used egg-swapping experiments in which subjects were presented with either a conspecific egg or a heterospecific egg to compare the egg rejection responses of a frequently parasitised host, the white-plumed honeyeater (*Lichenostomus penicillatus*), with two less frequently parasitised hosts, dusky woodswallows (*Artamus cyanopterus*) and willie wagtails (*Rhipidura leucophrys*). As predicted, rejection rate increased as contrast between foreign egg and host clutch increased. Further, the major host showed greater between-clutch variation than the occasional hosts, and also rejected more similar-looking eggs. Contrary to predictions however, within-clutch variation was not lower in the major host, nor was it important in predicting the rejection rate of foreign eggs by the three host species.

Keywords: brood parasitism, coevolution, spectrophotometry, egg mimicry, clutch variation, egg discrimination, arms race.

Introduction

Brood parasites lay their eggs in the nests of other species, abandoning their young entirely to the care of the host. The host pays a heavy cost for the upbringing of the foreign chick and as a result evolves defences which are specifically tuned to reduce parasitism, the most notable of which is the discrimination and rejection of foreign eggs (Davies, 2000). However, parasites evolve counter-defences such as egg mimicry, aimed at evading the host's defences.

When cuckoos lay highly mimetic eggs, it has been suggested that some hosts respond by evolving egg colours and patterns that look different from the parasitic eggs in order to help in egg identification. This is based on two hypotheses. The first is that hosts maximise their chances of detecting a cuckoo egg by reducing variation within their clutches — if eggs within a clutch look similar to one another, a foreign egg would appear more distinctive (Davies & Brooke, 1989; Stokke et al., 1999; Kilner, 2006). The second theory argues that increased variation between clutches in a population decreases cuckoo egg mimicry because when clutches in a population look different from each other it is harder for a parasite to mimic any particular clutch (Swynnerton, 1918; Davies & Brooke, 1989; Honza et al., 2004; Kilner, 2006).

Many previous tests of these hypotheses are limited by the use of subjective human assessments of clutch variation. Birds have one of the richest capacities for colour vision within vertebrates (Goldsmith, 1990; Kelber et al., 2003; Hart & Hunt, 2007), allowing many of them to see ultraviolet light (300–400 nm), which humans cannot see. One way to assess egg appearance objectively and independently of human colour perception is to use reflectance spectrophotometers sensitive to ultraviolet light in order to encompass the entire bird-visible waveband (ca. 300–700 nm, compared to 400–700 nm for humans, Bennett & Cuthill, 1994).

Previous studies have found evidence both for and against these hypotheses of clutch variation. Of those studies that have incorporated the use of reflectance spectrophotometry, two have supported the hypothesis that parasitism reduces within-clutch variation (Avilés & Møller, 2003; Lahti, 2005). Further, in one species the likelihood of foreign egg rejection increased with decreasing within-clutch variation (Polačiková et al., 2007). However, two studies yielded the opposite result: females who rejected foreign eggs actually had higher within-clutch variation than acceptors (Avilés et al., 2004;

Cherry et al., 2007). The only spectrophotometric study that has tested the effects of parasitism on clutch variation between females in a population (Lahti, 2005) found support for the hypothesis that parasitism increases between-clutch variation.

In this study we investigated the effects on host defences of parasitism by an Australian generalist parasite, the pallid cuckoo, *Cuculus pallidus*. This obligate brood parasite is recorded to have exploited over 100 different species of passerines, though all major hosts belong to the Family Meliphagidae (honeyeaters, Brooker & Brooker, 1989), and it lays eggs that mimic the eggs of its major hosts (Starling et al., 2006). We used reflectance spectrophotometry and egg-swapping experiments to assess the effects of pallid cuckoo parasitism on both the foreign egg rejection abilities and the extent of within-clutch and between-clutch variation of three host species: a 'major' pallid cuckoo host, the white-plumed honeyeater (*Lichenostomus penicillatus*), and two 'occasional' hosts, dusky woodswallows (*Artamus cyanopterus*) and willie wagtails (*Rhipidura leucophrys*) (Brooker & Brooker, 1989).

Methods

Study species selection

We selected species to study in the field based on the frequency of parasitism drawn from Brooker and Brooker's (1989) classification of cuckoo hosts. We aimed to compare a 'major' host with two less frequently parasitised 'occasional' hosts to determine whether rate of parasitism affects foreign egg rejection abilities.

The white-plumed honeyeater was selected as it is the most common pallid cuckoo host within the Australian Capital Territory (south-eastern Australia) (Taylor, 1992), and it was the most common host species observed rearing pallid cuckoo nestlings and fledglings on our study sites. Further, pallid cuckoo eggs laid in white-plumed honeyeater nests are highly accurate mimics of the host eggs (Starling et al., 2006). We selected two occasional hosts, dusky woodswallows and willie wagtails, based on the following criteria. First, they are classified as biological hosts of pallid cuckoos, but not as major hosts in south-eastern Australia by Brooker and Brooker (1989). Second, they breed commonly in the ACT (Taylor, 1992). Third, we

took into account egg size, since in the experiments in which we swapped eggs between the three host species we were interested in testing egg discrimination based on egg colour, but not size — eggs that are substantially larger than the foster bird's eggs are often rejected (Davies & Brooke, 1988; Langmore et al., 2003). Both dusky woodswallows (range of length × range of width = $(21.6–23.8) \times (16.3–18.0)$ mm) and willie wagtails $((17.5–21.3) \times (14.2–16)$ mm) have eggs that are similar in size to that of the major host $((19.3–21.8) \times (14.2–15.7)$ mm, Higgins et al., 2001; Higgins et al., 2006). Pallid cuckoo eggs do not mimic those of willie wagtails (human classification: Brooker & Brooker, 1989; spectrophotometric comparison: Starling, 2005), and to our eyes they do not appear to resemble those of dusky woodswallows (Plate 2 in Landstrom, 2008). The egg discrimination abilities of white-plumed honeyeaters were also tested using fuscous honeyeater (*Lichenostomus fuscus*) eggs. Fuscous honeyeaters also lay eggs of similar size $((18.3–21.3) \times (14.0–15.20)$ mm, Higgins et al., 2001) to white-plumed honeyeaters. No experiments were performed on fuscous honeyeaters.

The egg colours of these four host species could be successfully distinguished from one another using discriminant function analysis (DFA) (Figure 1), confirming that conspecific eggs were of relatively low contrast to one another, whereas heterospecific eggs were of high contrast.

Study areas

The study sites included three locations within the ACT: Campbell Park (35°16′S, 149°10′E), Gungahlin Hill Nature Reserve (35°12′S, 149°06′E) and Lake Ginninderra (35°13′S, 149°04′E), as well as two points in Namadgi National Park (35°44′S, 148°59′E; 35°45′S, 148°58′E). All sites comprised native eucalypt woodland except for Lake Ginninderra, which was a landscaped lakeshore.

Measurements

Spectral reflectance measurements of both transferred eggs and experimentally parasitised clutches were taken using an Ocean Optics USB2000 spectrophotometer and PX-2 pulsed xenon light source using OOIBASE software (Ocean Optics). Reflectance was measured using a narrow-ended UV-Vis unidirectional reflectance probe with a bevelled edge, held at a constant 45° angle to the surface of the egg, which illuminated areas approx. 1.5 mm in
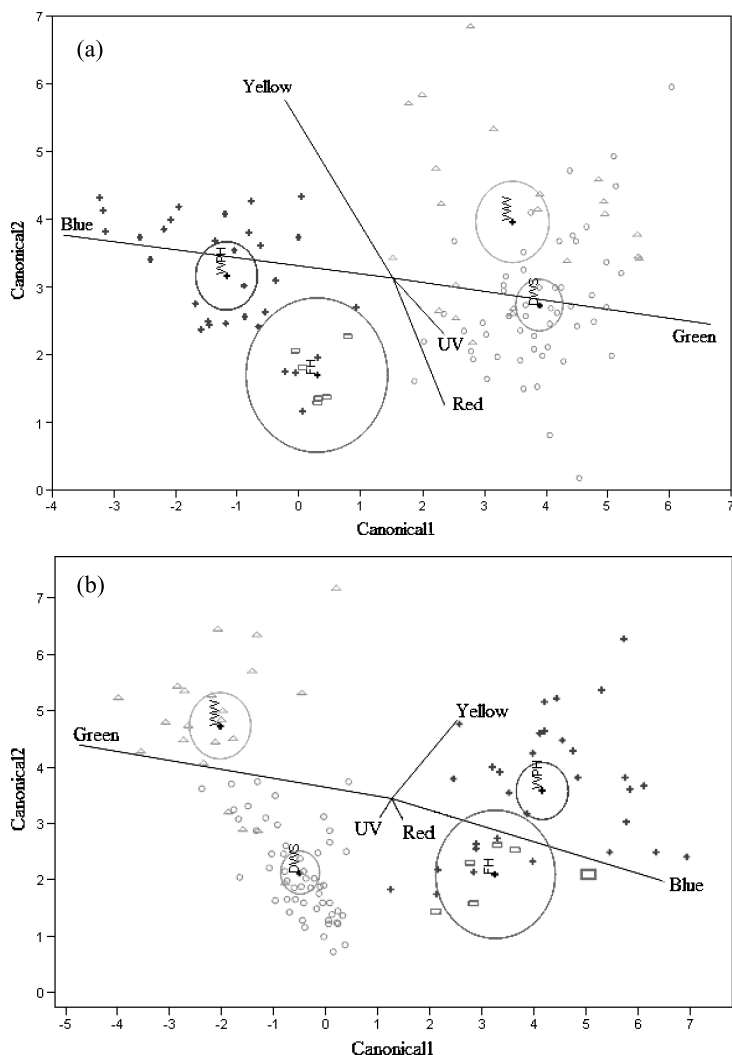
Figure 1. Discriminant function analysis of willie wagtail (WW, $N = 22$), dusky woodswallow (DWS, $N = 53$), white-plumed honeyeater (WPH, $N = 31$), and fuscous honeyeater (FH, $N = 6$) eggs from the field. (a) Side, background colour; (b) Ring, the maculated zone around the base of the egg. The mean reflectance spectra (Ring and Side, $N = 8$) of all eggs measured in the field were separated into five major chroma: UV 300–400 nm; blue 400–475 nm; green 475–550 nm; yellow 550–625 nm; red 625–700 nm. The chromatic means for each egg were calculated separately for Ring and Side measurements and entered into two DFAs (JMP 6.0). DFA labels each multivariate mean with a circle, and the size of the circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different have non-intersecting circles.

diameter. Measurements were relative to a standard white reference (WS-1 Diffuse Reflectance Standard) and to complete darkness. The spectrophotometer was re-standardised after each clutch. Integration time was set to 15 ms and reflectance was taken at 2-nm intervals over the range of bird-visible wavelengths (300–700 nm).

Four readings were taken in two regions on each egg, the 'Side' and the 'Ring', for a total of eight measurements. The regions were selected based on their distinct visual characteristics. 'Side' corresponds to the background colour, or the colour of the egg that makes up the majority of the egg appearance. These measurements were taken in the middle of the egg, somewhat above yet avoiding the region of the 'Ring'. 'Ring' refers to the concentration of dense speckling or region of darker colouration that usually appears near the base of maculated eggs.

Clutch manipulation experiments

Egg-recognition experiments were performed by transferring eggs between conspecific and heterospecific host nests to simulate parasitism by cuckoos with highly-mimetic eggs and cuckoos with poorly-mimetic eggs respectively. For conspecific swaps we transferred eggs between different females of the same species, whereas heterospecific swaps comprised transfers of eggs between females of different species. Conspecific eggs were typically closer in spectral reflectance (i.e., more 'mimetic') than heterospecific eggs. The amount of contrast was measured objectively with reflectance spectrophotometry.

Experiments were performed over two breeding seasons, between 2006 and 2008 (September through January 2007 and August through January 2008). Ideally, the nests were discovered during the building stage or during the egg laying stage. Many nests, however, were found during the incubation period. The likelihood of egg rejection appears to decline during the incubation period in some species (e.g., Moksnes et al., 1993; Welbergen et al., 2001), but not others (Moksnes et al., 1990; Jackson, 1998). In order to control for timing of parasitism, we calculated the day of laying or incubation on which the artificial parasitism event took place, either as the number of days since the first egg was laid, or if that was not known, by back-dating from the day the chicks hatched.

We followed the egg-rejection experiments described in Langmore et al. (2005). We warmed the foreign egg (if cold) by wrapping it in a tissue to

protect the surface and bringing it to approximately human body temperature (37°C) then placed it in the experimental nest. Unless an egg from that nest was used in another experiment, no additional egg was removed from the nest, since hosts do not appear to discriminate against having an 'extra' egg (Davies & Brooke, 1988; Jackson, 1998). Given that hosts tend to abandon nests that have been greatly reduced in egg number (Hill & Sealy, 1994), we did not remove an egg without replacement from nests containing only two eggs (which is common in honeyeaters). We left the foreign egg in the nest for five full days and if on the sixth day it was still being incubated we returned it to its original nest, if the original nest was still active. If the original nest was no longer active we placed the egg in a surrogate nest of the same species that contained eggs of roughly the same age. If no nests were available we saved the egg for the following experiment. If any of the eggs hatched, or if the nest was preyed upon before the sixth day, the experiment was excluded from the dataset.

For any individual host only one experiment was performed during each nesting attempt and no one type of experiment was performed more than once. This was to eliminate any chance of imprinting on the foreign egg type and artificially reducing rejection rate in subsequent experiments (Lotem et al., 1992), or of alerting the host to parasite susceptibility and artificially increasing rejection rate (Hauber et al., 2006).

Eggs were marked with a small dot at the flattened base with a non-toxic marker to distinguish the foreign egg, and also to identify the eggs when taking spectrophotometer readings. When a clutch no longer appeared translucent (>4 days incubation) we took reflectance spectrophotometer readings of each egg.

On the sixth day the experiment outcome was scored as either 'accepting' or 'rejecting' the foreign egg. An egg was considered accepted if it was undamaged and being incubated by the host. It was classified as rejected if the clutch was either abandoned (eggs cold with no apparent nesting activity) or if the foreign egg was missing or damaged.

Our dataset also includes the results of conspecific willie wagtail nest manipulation experiments conducted by Starling (2005) at the same study sites ($N = 8$), following the same methods. Experiments were conducted with the approval of the ANU Animal Experimentation Ethics Committee, protocol no. F.BTZ.02.06.

Statistical analysis

Principal components analysis

All reflectance spectra (Ring and Side, $N = 8$) for each egg measured (eggs measured $N = 124$, total spectra $N = 992$) were entered into a single principal components analysis (PCA), and the coefficients were plotted against wavelength to depict the variation in colour that was explained by each principal component. PCA is useful for evaluating variation in spectral data because it summarises the most important variation in multi-dimensional data sets for analysis. It extracts patterns (factors) that explain the most variation in the data. Following the methods of Avilés et al. (2006), Starling et al. (2006) and Cherry et al. (2007) the first three principal components (PC1, PC2, PC3) were calculated for each spectra for each egg, and the mean score of PC1, PC2 and PC3 was determined for each egg. GenStat 10th edition (2007) was used for all principal components analyses.

To calculate the degree of egg colour matching between the host clutch and foreign egg, the difference between the foreign egg and mean host score for each experiment was calculated separately for PC1, PC2 and PC3. The standard deviations of the mean values of all the eggs in a clutch were also calculated and used as measures of intraclutch variation in eggshell colouration for each of the first three principal components. Only clutches with three eggs were considered for this analysis, because number of eggs in the clutch could also influence the magnitude of the standard deviations.

What influences rejection rate?

To determine how different factors predict likelihood of foreign egg rejection, data were analysed with logistic regression using JMP 6.0 (2005). The host's response to the experimental egg (reject/accept) was the dependent variable, and independent variables included day of 'parasitism' (number of days from when the first host egg was laid to insertion of foreign egg), the rate of parasitism experienced by the host species ('common' for white-plumed honeyeaters and 'occasional' for dusky woodswallows and willie wagtails), foreign egg matching score (for PC1, PC2 and PC3), and amount of within-clutch variation (also for PC1, PC2, PC3). Each of the first three principal components was entered in a separate model (for example, PC1 for egg matching with PC1 for within-clutch variation), along with rate of parasitism and all two- and three-way interactions.

The effect of parasitism rate on clutch variation

If the degree of cuckoo parasitism influences the evolution of clutch variation in hosts, white-plumed honeyeaters, the major host, should exhibit greater between-clutch variation and less within-clutch variation than the occasional hosts. We compared within-clutch and between-clutch variation for the three species using pair-wise comparisons of variances. The mean reflectance spectra for each egg from the two species was entered into two separate principal components analyses (one for Ring, the other for Side) and the first three principal components were determined in each case. The resulting six PCs were entered separately into generalized linear models. Each clutch was given a separate nest identifier to account for having multiple eggs in a clutch, and each egg was identified separately within each clutch. Also included were the species of the eggs. The resulting accumulated analysis of variance yielded the amount of variance associated with within-clutch and between-clutch variation, and variance ratios (F statistics) were generated for all comparisons of interest. Differences between the mean squares were then tested using F statistics. The species predicted to have the higher amount of variation was set as the numerator, and that predicted to have the less variability was set as the denominator of the variance ratio. Of the two occasional hosts, willie wagtails suffer a higher rate of parasitism (Brooker & Brooker, 1989). Bonferroni corrections were applied to account for the use of each dataset in two comparisons. To determine if significance was more than we might expect to find by chance, we also tested the opposing hypothesis: that species with a higher rate of parasitism have more within-clutch variation.

In addition to using variance ratios, the effect of degree of parasitism on the amount of colour variability within clutches was analysed using least squares regression (JMP 6.0). The within-clutch variation (as measured by standard deviation) for each of the first three principal components was designated the dependent variable with rate of parasitism the independent variable.

Results

Do hosts reject foreign eggs?

The day of the nesting cycle in which the experimental egg was added did not influence the likelihood of rejection (accept/reject) ($N = 35$, $\chi_1^2 =$

Table 1. The number of conspecific and heterospecific eggs accepted and rejected in experimental trials.

Host species	Experimental egg				
	Willie wagtail	Dusky wood-swallow	Fuscous honeyeater	White-plumed honeyeater	Total rejected (%)
Willie wagtail	Reject: 0 Accept: 8	Reject: 1 Accept: 1	Reject: N/A Accept: N/A	Reject: 4 Accept: 0	36
Dusky woodswallow	Reject: 2 Accept: 0	Reject: 2 Accept: 12	Reject: N/A Accept: N/A	Reject: 4 Accept: 0	40
White-plumed honeyeater	Reject: 2 Accept: 0	Reject: 1 Accept: 1	Reject: 3 Accept: 0	Reject: 4 Accept: 4	71

Host species are shown in rows and the species of the egg added to the nests are shown in columns. No experiments were conducted on fuscous honeyeaters.

[*] Sample sizes are comparable to those of Soler et al. (2000) and Procházka & Honza (2003).

0.70, $p = 0.40$), so nests parasitised at different times were all included in the following analyses.

All three host species demonstrated an ability to discriminate and reject foreign eggs. White-plumed honeyeaters, the major hosts, were able to reject both heterospecific eggs and conspecific eggs. Dusky woodswallows and willie wagtails, the two occasional hosts, showed strong discrimination of heterospecific eggs, but were poor rejecters of conspecific eggs (Table 1; Figure 2).

What influences rejection rate?

We tested whether the independent variables 'egg-matching', 'within-clutch variation', and 'rate of parasitism' influenced the likelihood of rejection using a logistic regression. Three models were fitted, one for each principal component. PC1 (accounting for 77.75% of the variation) was mostly flat and explained much of the variation in brightness but also explained some variation in the longer wavelengths (Figure 3). PC2 explained 18.15% of the variation, and varied most in the longer wavelengths so would primarily describe variation in yellows, oranges, and reds, while PC3 explained 2.11% of the variation and showed the most variation in lower wavelengths, especially the short to medium wavelengths (UV through blue–green) (Figure 3). The only significant variables were the egg-matching scores for PC1
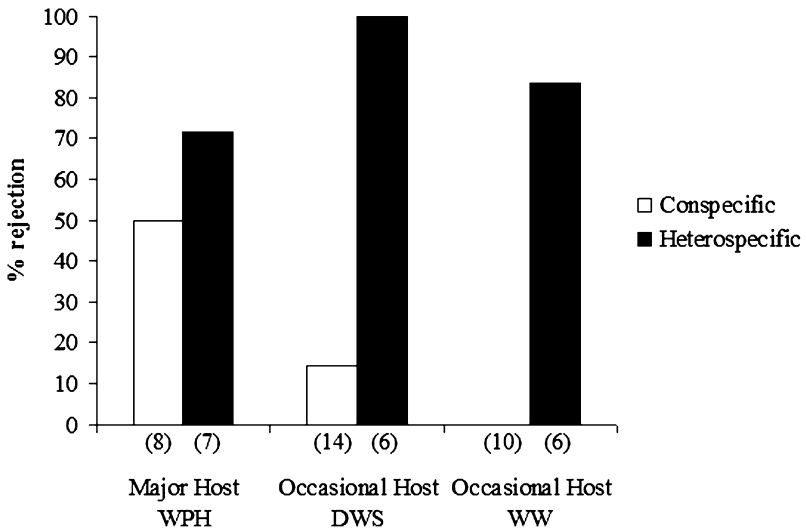
Figure 2. Response of major hosts (white-plumed honeyeaters) and occasional hosts (dusky woodswallows and willie wagtails) to foreign conspecific and heterospecific eggs. Sample sizes given in parentheses.

($\chi_1^2 = 9.24$, $p = 0.0024$) and PC2 ($\chi_1^2 = 4.81$, $p = 0.0282$), which suggests that rejection increases with increasing difference in luminance (PC1) and yellows–reds (PC2) between the host clutch and foreign egg.

The effect of parasitism rate on clutch variation

When eggs from the field were analysed using reflectance spectrophotometry, white-plumed honeyeaters and willie wagtails were found to have more between-clutch variation than dusky woodswallows in all variables measured, with the exception of PC3 for Side (Table 2). White-plumed honeyeaters tended to have more between-clutch variation than willie wagtails, but not significantly so.

No measures of within-clutch variation were significant, except PC3 for Side — white-plumed honeyeaters were more variable than dusky woodswallows. PC3 was mostly associated with variation in lower wavelengths from UV to green. Overall, the amount of within-clutch variation in these three species was found to be contrary to predictions, and followed the same pattern as between-clutch variation: white-plumed honeyeaters showed the highest variation, followed by willie wagtails, and dusky woodswallows had the least (Table 3). This was supported by regression analysis, which
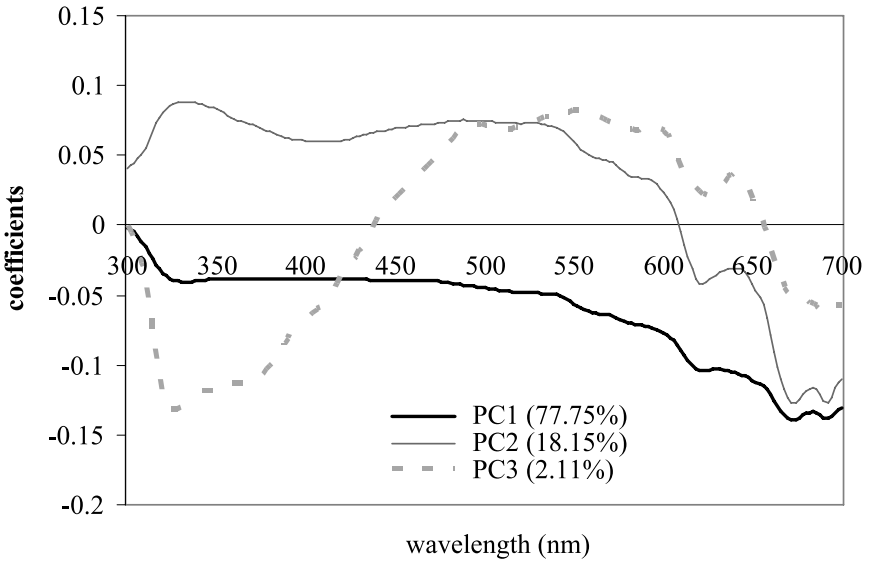
Figure 3. The coefficients of the first three principal components against wavelength; includes all measurements ($N = 8$) of each egg measured in the field (eggs measured $N = 124$, total spectra $N = 992$). Percent of variation explained by each PC is shown in parentheses.

Table 2. Differences between species in the amount of intraspecific between-clutch variation for the first three principal components.

	Ring	p-value	Side	p-value
DWS vs. WPH: $F_{8,16}$	PC1	0.056	PC1	<0.001*
	PC2	<0.001*	PC2	<0.001*
	PC3	0.013*	PC3	0.963
WW vs. WPH: $F_{8,6}$	PC1	0.460	PC1	0.064
	PC2	0.194	PC2	0.266
	PC3	0.439	PC3	0.932
DWS vs. WW: $F_{6,16}$	PC1	0.044	PC1	0.040
	PC2	<0.001*	PC2	<0.001*
	PC3	0.011*	PC3	0.165

Due to the use of each dataset in two independent tests, significance was assessed with a Bonferroni correction ($p < 0.025$). Willie wagtails are predicted to have marginally more between-clutch variation than dusky woodswallows. DWS, dusky woodswallow; WW, willie wagtail; WPH, white-plumed honeyeater.

* Significant p-value, indicating that the first species has lower between-clutch variation than the second species.

Table 3. Differences between species in the amount of within-clutch variation for the first three principal components.

	Ring	*p*-value	Side	*p*-value
DWS vs. WPH: $F_{34,12}$	PC1	1.000*	PC1	0.999*
	PC2	1.000*	PC2	0.880
	PC3	0.931	PC3	**0.019**
WW vs. WPH: $F_{16,12}$	PC1	0.952	PC1	0.993*
	PC2	0.999*	PC2	0.266
	PC3	0.911	PC3	0.629
DWS vs. WW: $F_{30,16}$	PC1	0.982*	PC1	0.915
	PC2	0.661	PC2	0.930
	PC3	0.791	PC3	0.855

Due to the use of each dataset in two independent tests, significance was assessed with a Bonferroni correction ($p < 0.025$). Variables significant in the predicted direction are shown in bold, and variables significant in the opposite direction are marked with asterisks. Willie wagtails are predicted to have marginally less within-clutch variation than dusky woodswallows. DWS, dusky woodswallow; WW, willie wagtail; WPH, white-plumed honeyeater. A significant *p*-value indicates that the first species has higher within-clutch variation than the second species.

demonstrated that within-clutch variation (the standard deviation of PC1, PC2 and PC3) increased with higher rates of parasitism. Greater rates of parasitism were associated with increased within-clutch variation in PC1 ($N = 27$, $F_1 = 14.08$, $p = 0.0009$), which mainly explains variation in luminance, and PC2 ($N = 27$, $F_1 = 13.27$, $p = 0.0012$), which mainly explains variation in longer wavelengths, but not PC3 ($N = 27$, $F_1 = 1.74$, $p = 0.1994$), which explains shorter wavelengths (Figure 3); the primary host had more within-clutch variation in PC1 and PC2 and a tendency to be more variable in PC3, while in the occasional hosts willie wagtails had somewhat more variation in PC1 than the less parasitised dusky woodswallows.

Discussion

This is one of the first studies to show that Australian cuckoo hosts can reject foreign eggs (see also Welbergen et al., 2001; Langmore et al., 2005) and the first study to test discrimination abilities in relation to clutch variation and parasitism rates in Australian cuckoo hosts.

As predicted by coevolutionary theory (Rothstein, 1990; Davies, 2000), not only were these species able to reject foreign eggs, but the major host showed a greater ability to discriminate eggs very similar to its own than the occasional hosts. White-plumed honeyeaters rejected 50% of conspecific eggs while the occasional hosts, dusky woodswallows and willie wagtails, rejected 14% and 0% of foreign conspecific eggs, respectively. The major factor influencing rejection of foreign eggs was the contrast in appearance between the host eggs and the foreign egg. Similarly, previous intraspecific studies (Procházka & Honza, 2003; Honza et al., 2004; Lovászi & Moskát, 2004; Stokke et al., 2004; Antonov et al., 2006) have found that the degree of contrast is a reliable indicator of egg rejection. In our study, contrasts in the first two principal components, which represent brightness and longer wavelengths, respectively, were the best predictors of rejection. This makes intuitive sense, because the birds were apparently basing their rejection decisions on the two characteristics of egg colouration that showed the most variability.

As predicted, the degree of between-clutch variation within each species increased with increasing rates of parasitism. This may explain why the major host was better able to discriminate conspecific eggs, and suggests that heavily parasitised hosts may have experienced selection for a higher level of between-clutch variation because this facilitates detection of mimetic cuckoo eggs. In a study conducted by Lahti (2005), populations of African village weaverbirds (*Ploceus cucullatus*) isolated from parasitic egg-mimicking diederik cuckoos (*Chrysococcyx caprius*) experienced a reduction in both between-clutch variation and within-clutch consistency, as measured using reflectance spectrophotometry. This suggests that high between-clutch variation is maintained through parasitism because it aids in foreign egg discrimination.

Contrary to theoretical predictions, within-clutch variation was not lower in the more heavily parasitised host. There are several possible explanations for this. First, the argument that reduced within-clutch variation should evolve in response to parasitism rests on the assumption that reduced within-clutch variation facilitates egg discrimination (Kilner, 2006), and this assumption was not supported here; within-clutch variation did not affect rejection. Second, the lack of influence of within-clutch variation on rejection was less surprising for the occasional hosts than for the major host, because theoretical models predict that with increasingly poor egg mimicry, within-clutch

variation becomes less important in determining the fitness of a rejecter strategy (Stokke et al., 2007). Third, some experimental evidence suggests that hosts do not compare the appearance of eggs within a clutch when choosing one for rejection, but instead rely on a memorized image of their own egg type (Lahti & Lahti, 2002). Finally, support for this hypothesis from other studies is, at best, mixed. Two of three previous intraspecific studies that used reflectance spectrophotometry to measure the amount of within-clutch variation of rejecters versus acceptors in a host population failed to support the hypothesis; these studies also found within-clutch variation to be higher, not lower, in rejecters (Avilés et al., 2004; Cherry et al., 2007), while the third study by Polačiková et al. (2007) was in support of the hypothesis. Two intraspecific spectrophotometric studies compared host populations that were either allopatric or sympatric with parasitic cuckoos (Avilés & Møller, 2003; Lahti, 2005), and supporting the hypothesis, both found within-clutch variation to be lower in parasitised populations. Additionally, Moskát et al. (2008) recently found that artificially increasing within-clutch variation by manipulating maculation led to lower rates of foreign egg rejection. Overall, our results of increased between-clutch variation, but no decrease in within-clutch variation in the major host, match those of two broadscale comparative analyses (Stokke et al., 2002; Kilner, 2006).

A possible extension of this argument is that the evolution of reduced within-clutch variation may be constrained by selection for increased between-clutch variation. If selection acts more strongly to drive increasing clutch variation between individuals than to lower variability within clutches, then perhaps the strong selection for mutations that allow an individual's egg to diverge from the population mean might also result in an associated increase in within-clutch variation. Some indirect support for this possibility comes from Kilner's (2006) comparative analysis, which shows a positive relationship between intraclutch variation and interclutch variation across host species.

References

Antonov, A., Stokke, B.G., Moksnes, A. & Røskaft, E. (2006). Egg rejection in marsh warblers (*Acrocephalus palustris*) heavily parasitized by common cuckoos (*Cuculus canorus*). — Auk 123: 419-430.

Avilés, J.M. & Møller, A.P. (2003). Meadow pipit (*Anthus pratensis*) egg appearance in cuckoo (*Cuculus canorus*) sympatric and allopatric populations. — Biol. J. Linn. Soc. 79: 543-549.

Avilés, J.M., Soler, J.J., Soler, M. & Møller, A.P. (2004). Rejection of parasitic eggs in relation to egg appearance in magpies. — Anim. Behav. 67: 951-958.

Avilés, J.M., Stokke, B.G., Moksnes, A., Røskaft, E., Åsmul, M. & Møller, A.P. (2006). Rapid increase in cuckoo egg matching in a recently parasitized reed warbler population. — J. Evol. Biol. 19: 1901-1910.

Bennett, A.T.D. & Cuthill, I.C. (1994). Ultraviolet vision in birds: what is its function? — Vision Res. 34: 1471-1478.

Brooker, M.G. & Brooker, L.C. (1989). Cuckoo hosts in Australia. — Aust. Zool. Rev. 2: 1-67.

Cherry, M.I., Bennett, A.T.D. & Moskát, C. (2007). Host intra-clutch variation, cuckoo egg matching and egg rejection by great reed warblers. — Naturwissenschaften 94: 441-447.

Davies, N.B. (2000). Cuckoos, cowbirds and other cheats. — T & AD Poyser, London.

Davies, N.B. & Brooke, M.D.L. (1988). Cuckoos versus reed warblers: adaptations and counter adaptations. — Anim. Behav. 36: 262-284.

Davies, N.B. & Brooke, M.D.L. (1989). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. — J. Anim. Ecol. 58: 225-236.

Goldsmith, T.H. (1990). Optimization, constraint, and history in the evolution of eyes. — Q. Rev. Biol. 65: 1990.

Hart, N.S. & Hunt, D.M. (2007). Avian visual pigments: characteristics, spectral tuning, and evolution. — Am. Nat. 169: S7-S26.

Hauber, M.E., Moskát, C. & Bán, M. (2006). Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. — Biol. Lett. 2: 177-180.

Higgins, P.J., Peter, J.M. & Cowling, S.J. (2006). Handbook of Australian, New Zealand and Antarctic Birds. — Oxford University Press, Melbourne, VIC.

Higgins, P.J., Peter, J.M. & Steele, W.K. (2001). Handbook of Australian, New Zealand and Antarctic Birds. — Oxford University Press, Melbourne, VIC.

Hill, D.P. & Sealy, S.G. (1994). Desertion of nests parasitized by cowbirds: have clay-coloured sparrows evolved an anti-parasite defence? — Anim. Behav. 48: 1063-1070.

Honza, M., Procházka, P., Stokke, B.G., Moksnes, A., Røskaft, E., Čapek Jr., M. & Mrlík, V. (2004). Are blackcaps current winners in the evolutionary struggle against the common cuckoo? — J. Ethol. 22: 175-180.

Jackson, W.M. (1998). Egg discrimination and egg-color variability. — In: Parasitic birds and their hosts (Rothstein, S.I. & Robinson, S.K., eds). Oxford University Press, New York, NY, p. 407-416.

JMP Version 7. (2005). SAS Institute, Cary, NC.

Kelber, A.M., Vorobyev & Osorio, D. (2003). Animal colour vision: behavioural tests and physiological concepts. — Biol. Rev. 78: 81-118.

Kilner, R.M. (2006). The evolution of egg colour and patterning in birds. — Biol. Rev. 81: 383-406.

Lahti, D.C. (2005). Evolution of bird eggs in the absence of cuckoo parasitism. — Proc. Natl. Acad. Sci. USA 102: 18057-18062.

Lahti, D.C. & Lahti, A.R. (2002). How precise is egg discrimination in weaverbirds? — Anim. Behav. 63: 1135-1142.

Landstrom, M.T. (2008). Coevolution between pallid cuckoos *Cuculus pallidus* and their hosts. — Masters thesis, The Australian National University, Canberra, ACT.

Langmore, N.E., Hunt, S. & Kilner, R.M. (2003). Escalation of a coevolutionary arms race through host rejection of brood parasitic young. — Nature 422: 157-160.

Langmore, N.E., Kilner, R.M., Butchart, S.H.M., Maurer, G., Davies, N.B., Cockburn, A., Macgregor, N.A., Peters, A., Magrath, M.J.L. & Dowling, D.K. (2005). The evolution of egg rejection by cuckoo hosts in Australia and Europe. — Behav. Ecol. 16: 686-692.

Lotem, A., Nakamura, H. & Zahavi, A. (1992). Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. — Behav. Ecol. 3: 128-132.

Lovászi, P. & Moskát, C. (2004). Break-down of arms race between the red-backed shrike (*Lanius collurio*) and common cuckoo (*Cuculus canorus*). — Behaviour 141: 245-262.

Moksnes, A., Røskaft, E., Braa, A.T., Korsnes, L., Lampe, H. & Pedersen, H.C. (1990). Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. — Behaviour 116: 64-89.

Moksnes, A., Røskaft, E. & Korsnes, L. (1993). Rejection of cuckoo (*Cuculus canorus*) eggs by meadow pipits (*Anthus pratensis*). — Behav. Ecol. 4: 120-127.

Moskát, C., Avilés, J.M., Bán, M., Hargitai, R. & Zölei, A. (2008). Experimental support for the use of egg uniformity in parasite egg discrimination by cuckoo hosts. — Behav. Ecol. Sociobiol. 62: 1885-1890.

Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Welham, S.J., Kane, A.F., Gilmour, A.R., Thompson, R., Webster, R. & Tunnicliffe Wilson, G. (2007). GenStat Release 10 Reference Manual. VSN International, Hemel Hempstead.

Polačiková, L., Honza, M., Procházka, P., Topercer, J. & Stokke, B.G. (2007). Colour characterstics of the blunt egg pole: cues for recognition of parasitic eggs as revealed by reflectance spectrophotometry. — Anim. Behav. 74: 419-427.

Procházka, P. & Honza, M. (2003). Do common whitethroats (*Sylvia communis*) discriminate against alien eggs? — J. Ornithol. 144: 354-363.

Rothstein, S.I. (1990). A model system for coevolution: avian brood parasitism. — Annu. Rev. Ecol. Syst. 21: 481-508.

Soler, J.J., Soler, M. & Møller, A.P. (2000). Host recognition of parasite eggs and the physical appearance of host eggs: the magpie and its brood parasite the great spotted cuckoo. — Etología 8: 9-16.

Starling, M. (2005). The effect of interspecific interactions on the breeding behaviour of the willie wagtail, *Rhipidura leucophrys*. — Honours thesis, The Australian National University, Canberra, ACT.

Starling, M., Heinsohn, R., Cockburn, A. & Langmore, N.E. (2006). Cryptic gentes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. — Proc. Roy. Soc. Lond. B: Biol. 273: 1929-1934.

Stokke, B.G., Moksnes, A. & Røskaft, E. (2002). Obligate brood parasites as selective agents for evolution of egg appearance in passerine birds. — Evolution 56: 199-205.

Stokke, B.G., Moksnes, A., Røskaft, E., Rudolfsen, G. & Honza, M. (1999). Rejection of artificial cuckoo (*Cuculus canorus*) eggs in relation to variation in egg appearance among reed warblers (*Acrocephalus scirpaceus*). — Proc. R. Soc. B 266: 1483-1488.

Stokke, B.G., Rudolfsen, G., Moksnes, A. & Røskaft, E. (2004). Rejection of conspecific eggs in chaffinches: the effect of age and clutch characteristics. — Ethology 110: 459-470.

Stokke, B.G., 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.

Swynnerton, C.F.M. (1918). Rejections by birds of eggs unlike their own: with remarks on some of the cuckoo problems. — Ibis: 127-154.

Taylor, M. (1992). Birds of the Australian capital territory, an atlas. — Canberra Ornithologists Group and National Capital Planning Authority, Canberra, ACT.

Welbergen, J., Komdeur, J., Kats, R. & Berg, M. (2001). Egg discrimination in the Australian reed warbler (*Acrocephalus australis*): rejection response toward model and conspecific eggs depending on timing and mode of artificial parasitism. — Behav. Ecol. 12: 8-15.