

# Egg mimicry by the pacific koel: mimicry of one host facilitates exploitation of other hosts with similar egg types

Virginia E. Abernathy<sup>a,c</sup>, Jolyon Troscianko<sup>b</sup> and Naomi E. Langmore<sup>a</sup>

<sup>a</sup>Ecology and Evolution, Research School of Biology, Australian National University, Acton, ACT

<sup>b</sup>Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, UK

<sup>c</sup>Corresponding author: Virginia Abernathy, 3135 Elliot Ave, Dayton, OH 45420 USA, email: [vabernathy123@gmail.com](mailto:vabernathy123@gmail.com), telephone: +1-937-409-2611

## ABSTRACT

When brood parasites exploit multiple host species, egg rejection by hosts may select for the evolution of host-specific races, where each race mimics a particular host's egg type. However, some brood parasites that exploit multiple hosts with the ability to reject foreign eggs appear to have only a single egg type. In these cases, it is unclear how the parasite egg escapes detection by its hosts. Three possible explanations are: (i) host-specific races are present, but differences in egg morphology are difficult for the human eye to detect; (ii) the brood parasite evolves a single egg type that is intermediate in appearance between the eggs of its hosts; (iii) or the parasite evolves mimicry of one of its hosts, which subsequently allows it to exploit other species with similar egg morphology. Here we test these possibilities by quantifying parameters of egg appearance of the brood-parasitic Pacific Koel (*Eudynamys orientalis*) and seven of its hosts. Koel eggs laid in the nests of different hosts did not show significant differences in colour or pattern, suggesting that koels have not evolved host-specific races. Koel eggs were similar in colour, luminance and pattern to the majority of hosts, but were significantly more similar in colour and luminance to one of the major hosts than to two other major hosts, supporting hypothesis (iii). Our findings suggest that mimicry of one host can allow a brood parasite to exploit new hosts with similar egg morphologies, which could inhibit the evolution of host defences in naïve hosts.

**Keywords:** brood parasitism, coevolution, egg mimicry, *Eudynamys orientalis*, Pacific Koel

## INTRODUCTION

An avian obligate brood parasite lays its eggs in the nests of other species and never provides parental care. This behaviour can be very costly for the host that raises the parasitic young, especially when all host young are killed or outcompeted by the brood parasite chick (Davies 2000). Therefore, there should be strong selection for hosts to evolve defences to reduce this cost. One such effective defence is egg rejection, where the parasitic egg is removed from the nest, buried under nesting material, or abandoned by the host if removal is not possible (Rothstein 1990; Hosoi and Rothstein 2000). As a result of rejection of non-mimetic eggs by hosts, many brood parasitic species have evolved a mimetic egg that is difficult to distinguish from the host egg (Davies 2011).

Egg mimicry becomes more complicated when a brood parasite exploits different host species simultaneously. In several parasitic cuckoos, rejection of foreign eggs by multiple hosts species has selected for host-specific cuckoo races, with each race specialising on a single host and laying an egg type matching that of its favoured host (Davies 2000; Antonov et al. 2010). In some cases, this evolutionary arms race escalates to a further stage, in which a single host species can evolve highly polymorphic eggs to escape mimicry, which in turn selects for polymorphic eggs in the brood parasite (Yang et al. 2010; Spottiswoode and Stevens 2011; Spottiswoode and Stevens 2012; Yang et al. 2013; see also Medina et al. 2016). Given this potential for highly accurate egg mimicry, it is puzzling that some brood parasites exhibit only a single egg type, despite exploiting multiple hosts that reject foreign eggs. Several explanations for this phenomenon have been proposed. First, when the host species have similar-looking eggs, a brood parasite could exhibit host-specific races that are cryptic to human observers, but are detectable to the host species viewing the eggs (Starling et al. 2006). Such evidence of cryptic races has been brought to light in Pallid Cuckoos (*Cuculus pallidus*) through objective quantification of egg colour (Starling et al. 2006). Second, if hosts exhibit low rates of egg rejection or poor egg discrimination, a brood parasite may evolve an intermediate egg type that appears moderately similar to all of its hosts, but is not a close mimic of any particular host egg type (Feeney et al. 2014). Third, a brood parasite could evolve mimicry of one host species, which then allows it to exploit other host species with similar egg types.

We test these possibilities in the Pacific Koel (*Eudynamys orientalis*; hereafter “koel”), a brood parasitic cuckoo in Northern and Southeast Australia that exploits five primary hosts, as well as several minor hosts, depending on the geographic location (Brooker and Brooker 1989; Brooker and Brooker 2005; Abernathy and Langmore 2017). The primary hosts include the Little Friarbird (*Philemon citreularis*), Noisy Friarbird (*Philemon corniculatus*), Australasian Figbird (*Sphecotheres vieilloti*), and Magpie-lark (*Grallina cyanoleuca*; Crouther and Crouther 1984; Brooker and Brooker 1989; Gosper 1997). In addition, in the 1930s-1970s, the koel adopted a new host, the Red Wattlebird (*Anthochaera carunculata*), which is now the primary host in some regions of New South Wales (NSW) and the Australian Capital Territory (ACT; Brooker and Brooker 2005; Abernathy and Langmore 2017). While some of these host species do live in sympatry, the fact that different geographic locations have different primary hosts (e.g. Red Wattlebirds are the primary host in the ACT and parts of NSW, even though Magpie-larks and Noisy Friarbirds both reside in these areas), suggests that individual female koels probably specialise on a single host species, though there have been no previous studies on koel laying behaviour.

Our previous work shows that when spotted model eggs with a similar ground colour to host eggs were placed in Noisy Friarbird and Magpie-lark nests, these hosts showed high rates of egg ejection (94%,  $N = 17$ ; 86%,  $N = 21$ , respectively; Abernathy 2017). By contrast,

the new host, the Red Wattlebird, is a poor egg rejecter (4% of spotted model eggs rejected,  $N = 25$ ) and so would not select for mimetic koel eggs (Abernathy 2017). The egg rejection abilities of the other two primary hosts are unknown. Egg rejection by Noisy Friarbirds and Magpie-larks would select for mimicry of these hosts' eggs by koels, yet koels have been described anecdotally as having one egg type, which closely matches the size, colour and pattern of both the major friarbird hosts, but is moderately different from Magpie-lark and Australasian Figbird eggs (Brooker and Brooker 1989; see also Beruldsen 1980). The apparent imperfect mimicry of Magpie-lark eggs is puzzling, given the high rate of rejection of foreign eggs in this species (Abernathy 2017). We quantify and compare egg colour, luminance, pattern and volume of koel and host eggs using reflectance spectrometry, digital photography and avian visual modelling. If hypothesis (i) is correct, we predict that we will find evidence of different egg types in koels, indicating the existence of host-specific races. If hypothesis (ii) is correct, we expect to find the morphological attributes of koel eggs to be intermediate among all of their hosts. Finally, if hypothesis (iii) is correct, koel eggs will be most similar to one host species, but the eggs of the other hosts will be morphologically similar. Given the lack of egg rejection by Red Wattlebirds and the recent interaction with this host, we predict that koel eggs will be relatively poor mimics of Red Wattlebird eggs.

## METHODS

We assessed egg mimicry in the koel using objective measurements of cuckoo and host egg appearance to determine from a bird's visual perspective if (i) koels exhibit host races, (ii) which host species have eggs that appear the most similar to the koel and (iii) how similar host species eggs appear to one another. From 2013-2015 we took measurements of egg colour, luminance, pattern and size of parasitised and unparasitised clutches of the koel's four traditional major hosts (Noisy Friarbird, Little Friarbird, Australasian Figbird and Magpie-lark), the new major host (Red Wattlebird) and two minor hosts (Blue-faced Honeyeater *Entomyzon cyanotis* and Olive-backed Oriole *Oriolus sagittatus*; Brooker and Brooker 1989) from the Australian National Wildlife Collection, CSIRO, ACT (Table 1; Fig. 1; Supplementary material Appendix 1 and 2). We also measured fresh Noisy Friarbird, Magpie-lark, Red Wattlebird and koel eggs from the field in Canberra, ACT and Western Sydney, NSW, Australia (Supplementary material Appendix 1 and 2). All fresh eggs and museum eggs were combined together for the egg pattern and egg size analyses, but for the colour analysis we separated fresh eggs from museum eggs, as spectra of eggs with contents appeared darker than blown eggs (VEA pers. observ.). Even though egg pigmentation has been shown to degrade over time, the main chromas that appear to be affected by museum storage include the blue-green and UV chromas (Starling et al. 2006; Cassey et al. 2010; Cassey et al. 2012). However, this is unlikely to have a major effect on our results, as all of the hosts in this study are thought to possess a VS-visual system (Ödeen and Håstad 2010; Ödeen et al. 2011; see below), which is the visual model we used when analysing the data, and all but one species in this study possess pink-beige coloured eggs (Fig. 1). Using the VS-visual system in the analysis would have excluded most of the reflectance data from the UV chroma, and pink-beige eggs would already show a low reflectance in the blue-green chroma. Therefore, in order to include some eggs from the present day in the museum egg colour analysis, a subset of fresh Red Wattlebird and koel eggs that were abandoned by the parents were collected, blown and measured again (Table 1; see also Spottiswoode and Stevens 2012).

From the museum, we measured every parasitised clutch, which included some host species that were only parasitised once or twice in the collection (Supplementary material Appendix 1). Host eggs from these clutches were only used in one egg size analysis where

host species was not a variable. For all analyses, every host egg from a clutch was measured (if possible) and an average for each host clutch was calculated. Each koel egg was treated independently, even if two eggs came from the same nest, as female koels would be unlikely to lay twice in the same nest because the first koel nestling to hatch would evict all other eggs and young from the nest (Higgins 1999).

### *Measurement and Analysis of Egg Colour, Luminance and Pattern*

We took objective measurements of the colour and luminance of eggs by measuring their spectral reflectance from 300-700 nm using an Ocean Optics Jaz spectrophotometer, a narrow ended UV-Vis unidirectional Ocean Optics QR400-7-SR reflectance probe with a 5 mm diameter, and an Ocean Optics WS-1 reflectance standard following the methods of Feeney et al. (2014). A white and dark reference were taken before each new egg was measured. Measurements of eggs in the field were taken under a black cloth to reduce noise from ambient light. The black cloth was not used when taking measurements inside the museum, as lighting conditions were more consistent. Eggs were divided into three regions, the cap, middle, and blunt end, and three measurements were taken in random areas in each of these regions. These nine measurements were averaged together to obtain an average spectral reflectance for each egg. Some measurements did include spot colour as well as background colour, but due to the small size of the spots, measuring these colours separately would have been difficult.

We used avian visual modelling to determine how similar eggs appear to one another chromatically (colour) and achromatically (luminance) from a bird's visual perspective. We used the violet-sensitive visual system (VS) known in the peafowl (*Pavo cristatus*) in our analyses, as members of two families represented by six of the host species in our study (Meliphagidae and Oriolidae), including the genera *Oriolus* and *Philemon*, all possess a VS system (Ödeen and Håstad 2010; Ödeen et al. 2011). While the Magpie-lark visual system has not been verified, the majority of species in the Corvoidea, which includes Magpie-larks, have a VS system (Ödeen et al. 2011). We calculated the cone stimulation (photon catch) values for each of the four avian colour cones (violet-sensitive, VS, shortwave-sensitive, SWS, mediumwave-sensitive, MWS and longwave-sensitive, LWS) and for the double cones (luminance) using the pavo package (Maia et al. 2013) in the R Statistical package (R Core Team 2016).

To measure egg pattern characteristics, we took a single photograph of each egg on a 16% grey standard kodak card using an EOS Kiss X5 Canon camera with a 100 mm f/2.8 macro lens. An Inca i3150 Lightweight Tripod was used to stabilise the camera and keep the lens at a constant distance from the egg. Objective image analysis was performed using the multispectral image calibration and analysis toolbox (Troscianko and Stevens 2015), for ImageJ (Rasband 1997-2014). The toolbox performs image calibration, ensuring linear reflectance images that control for lighting changes are used for image processing. Pattern analysis was performed using standard bandpass methods on the camera's green reflectance channel as this most closely approximates bird double cone peak sensitivities (e.g. see Spottiswoode and Stevens 2010). We calculated pattern energy spectrums for each egg using a size range of 2-512 px with a 50 px/mm scale. We averaged the egg pattern energies of all eggs within a clutch across each pixel size to obtain an average pattern energy spectrum for each host clutch.

To determine if koels have host-specific races in colour or pattern, we ran a discriminant function analysis on both parameters. For egg colour, we only included the

museum egg dataset, using the four photon catch values for the VS, SWS, MWS and LWS cones as covariates. For pattern we used the pattern energy at each pixel size (2.0, 2.8, 4.0, 5.7, 8.0, 11.3, 16.0, 22.6, 32.0, 45.3, 64.0, 90.5, 128.0, 181.0, 256.0, 362.0 and 512.0) as covariates. Koel eggs were grouped according to the host species' clutch in which they were found. For this analysis, we included host eggs from all seven host species, but we did not include koel eggs from Olive-backed Oriole nests, as this host had only four parasitised clutches. Results from the egg colour discriminant function analysis showed no significant differences between any of the koel groups except for the koel eggs in Red Wattlebird clutches, which were only similar to koel eggs in Australasian Figbird nests, but this difference is unlikely to be detectable by hosts (see Results, below; Fig. 2a). Likewise, the egg pattern discriminant function analysis showed no significant differences between any of the koel groups (see Results, below; Fig 2b). As there was no evidence to support hypothesis (i), that koels have host-specific races, we pooled all koel eggs together for the tests of hypotheses (ii) and (iii), comparing each koel egg to every host clutch in the dataset, regardless of the host species' clutch in which the koel egg was found.

For both the fresh and museum egg datasets, we compared colour and luminance of every koel egg to every host clutch and every host clutch to every other host clutch, which provided an output of just-noticeable-differences (JNDs) (following Vorobyev and Osorio 1998). JNDs are relative values where larger values indicate two eggs are more discriminable from each other than two eggs with smaller JNDs. In theory, a  $JND < 1$  indicates two eggs are indistinguishable, while a JND between 1-3 indicates two eggs are barely distinguishable in ideal lighting conditions and  $JNDs > 3$  indicate two eggs should be easily distinguishable in good lighting conditions (Siddiqi et al. 2004). The average chromatic (colour) and achromatic (luminance) JNDs between each koel egg or host clutch and every other host clutch were used in the statistical analyses. In the same way, we compared egg pattern between every koel egg or host clutch and every other host clutch by finding the absolute difference in pattern energies at each pixel size between each egg or clutch and then calculating the total pattern energy difference. We used the average absolute difference for each koel egg and host clutch in our statistical analysis.

The colour and luminance of Red Wattlebird eggs that were blown by us were also compared to Red Wattlebird eggs from the museum and the colour and luminance of koel eggs blown by us were compared to koel eggs from the museum using JND analyses to test whether the freshness of eggs influenced colour measurements. The average chromatic and achromatic JNDs for Red Wattlebirds and koels were low (Red Wattlebirds:  $0.92 \pm 0.32$  and  $1.26 \pm 0.66$ , respectively; koels:  $0.79 \pm 0.29$  and  $0.92 \pm 0.49$ , respectively), indicating that our blown Red Wattlebird and koel eggs would be difficult for a bird to distinguish from museum blown eggs. Because the discriminant function analysis for egg colour found a statistical difference between koel eggs laid in Red Wattlebird nests and koel eggs laid in four other host nests, we performed a JND analysis to determine if this difference could be detected by the hosts. For this analysis, we only compared koel eggs from Red Wattlebird nests to koel eggs from Magpie-lark, Noisy Friarbird, Little Friarbird and Blue-faced Honeyeater nests.

### *Measurement and Analysis of Egg Size*

We measured the length and width (mm) of each egg using Vernier callipers and then estimated volume (ml) using Narushin's (2005) formula:  $V = (0.6057 - 0.0018B)LB^2$ , where  $V$  = volume ( $\text{mm}^3$ ),  $B$  = breadth (mm), and  $L$  = length (mm). To determine if there were differences in egg size between the seven hosts species and the koel we performed an



ANOVA with Tukey HSD tests. To determine if host egg size could predict koel egg size, we ran two linear models (LMs). The first included the year the clutch was collected, the geographic region the clutch was from (NSW or QLD) and the host species as independent variables. We excluded any host species that had less than five parasitised clutches in the collection. For the second LM, we included year, geographic region, and the average egg volume for the parasitised host clutch as independent variables. For this model, we included every clutch where the host eggs had been measured, regardless of how many times a particular species had been parasitised. Sample sizes prevented the modelling of interaction terms.

### *Statistical Analysis*

Shapiro-Wilk normality tests indicated that most of the JND comparison groups for both the fresh and museum egg datasets, as well as most of the egg pattern comparison groups were not normal and a log transformation was not sufficient to make them normal (Shapiro-Wilk,  $P < 0.05$ ). Therefore, we performed nonparametric Kruskal-Wallis tests for these datasets and used nonparametric multiple comparison Dunn tests to determine which groups were significantly different. We used the pavo package (Maia et al. 2013) in the R Statistical Package (R Core Team 2016) to calculate JNDs and report average JNDs  $\pm$  standard deviations. Normality tests and all egg size analyses were performed in the R Statistical Package (R Core Team 2016) and the discriminant function analyses, Kruskal-Wallis and post-hoc tests were run in JMP 12.0.1. We used an alpha value of 0.05. For each LM non-significant terms were dropped sequentially until only significant terms remained.

### *Ethical Note*

This project was approved by and conducted in accordance with the Australian National University Animal Experimentation Ethics Committee: protocol number A2013/20. Permits from the Territory and Municipal Services of the ACT (license number: LT2013678) and of the Office of Environment and Heritage of the NSW National Parks and Wildlife Service (license number: SL101349) were obtained to conduct scientific experiments in Canberra and Western Sydney. Fresh eggs were measured as quickly as possible, typically at least one day after the final egg had been laid to ensure eggs were strong enough for measuring, and returned to nests promptly after measuring. Eggs from the field were only collected if it was clear the parents had abandoned the nest.

## RESULTS

### *Do Koels Have Host-specific Races?*

Koel egg colour did not separate into distinct groups based on host species, but instead were clustered together (Fig. 2a). Koel eggs laid in Red Wattlebird nests were slightly different from other koel eggs, only overlapping in colour with koel eggs from Australasian Figbird nests (Fig. 2a). However, this difference is unlikely to be detectable by hosts, as a JND analysis indicated that koel eggs laid in Red Wattlebird nests would not be discriminable from koel eggs laid in the nests of the four other hosts when seen through the eyes of a bird (average chromatic JND:  $0.85 \pm 0.31$ ; average achromatic JND:  $0.98 \pm 0.53$ ). Koel egg colours differed significantly from the egg colours of all the other hosts, except for Noisy

Friarbird and Red Wattlebird egg colour, and there was a slight overlap in colour between koel eggs laid in Red Wattlebird nests and Blue-faced Honeyeater eggs (Fig. 2a). The eggs of Noisy Friarbirds and Red Wattlebirds did not differ significantly in colour, but the egg colours of all other host species were significantly different from one another (Fig. 2a).

There were no significant differences in the patterns of koel eggs laid in the nests of different hosts and all koel eggs showed a similar pattern to Noisy Friarbird, Little Friarbird and Australasian Figbird eggs (Fig. 2b). Red Wattlebird eggs had a similar pattern to four koel groups and Blue-faced Honeyeater eggs had a similar pattern to two koel groups (Fig. 2b).

### *Which Hosts Are Koel Eggs Mimicking?*

Similar results were obtained using the JND analysis method when koel eggs were not separated by host species. Koel eggs were indistinguishable in colour from Noisy Friarbird eggs (average JNDs:  $0.93 \pm 0.26$ ) and barely distinguishable from Red Wattlebird eggs (average JNDs:  $1.03 \pm 0.31$ ). However, in this analysis koel eggs were also indistinguishable from Olive-backed Oriole eggs (average JNDs:  $0.86 \pm 0.19$ ). Average chromatic JNDs were between 1.10-1.92 for all other hosts compared to koels, indicating koel eggs are just distinguishable in colour from these hosts' eggs in good lighting conditions (Fig. 3). Koel eggs were significantly more discriminable in colour from Little Friarbird eggs than from eggs of all other hosts (Kruskal-Wallis:  $\chi^2_{27} = 762.21$ ,  $P < 0.001$ ; Fig. 3). Koel eggs had significantly more similar luminance to all other hosts (JNDs between 1.18-1.99) than to the Australasian Figbird (average JNDs:  $3.87 \pm 0.96$ ) and Little Friarbird (average JNDs:  $4.80 \pm 0.95$ ; Kruskal-Wallis:  $\chi^2_{27} = 1070.77$ ,  $P < 0.001$ ), but were most similar in luminance to Blue-faced Honeyeater, Red Wattlebird and Noisy Friarbird eggs (Fig. 3).

Fresh koel egg colour and luminance were equally similar to the eggs of all three hosts measured in the field (colour: Kruskal-Wallis:  $\chi^2_5 = 65.22$ ,  $P < 0.001$ ; Dunn tests:  $P > 0.05$  for all tests; luminance: Kruskal-Wallis:  $\chi^2_5 = 17.22$ ,  $P = 0.004$ ; Dunn tests:  $P > 0.05$  for all tests; Fig. 4) and should be just distinguishable from all three host eggs in colour and luminance in good lighting conditions (average chromatic JNDs between 1.13-1.51; average achromatic JNDs between 2.10-2.24).

Koel egg pattern was significantly more similar to the egg pattern of Noisy Friarbirds, Little Friarbirds, Blue-faced Honeyeaters, Australasian Figbirds and Red Wattlebirds than to Magpie-lark and Olive-backed Oriole egg pattern (Kruskal-Wallis:  $\chi^2_{27} = 1009.93$ ,  $P < 0.001$ ; Fig. 5).

### *How Similar Are Host Eggs to One Another?*

The egg colours of Blue-faced Honeyeaters, Noisy Friarbirds and Red Wattlebirds were indistinguishable from one another (all average JNDs  $< 1$ ). Olive-backed Oriole egg colour was just distinguishable from Noisy Friarbird (average JND:  $1.06 \pm 0.46$ ) and Australasian Figbird egg colour (average JND:  $1.05 \pm 0.51$ ). All other average chromatic JNDs comparing host egg colours were below three, with the majority below two, indicating all host eggs were either indistinguishable or just distinguishable from one another in good lighting conditions (Fig. 3). However, egg colour of both the Australasian Figbird and Olive-backed Oriole was significantly more discriminable from Little Friarbird egg colour than from the egg colour of all other species (Kruskal-Wallis:  $\chi^2_{27} = 762.21$ ,  $P < 0.001$ ; Fig. 3). While none of the hosts'

eggs were indistinguishable in luminance, the majority of average achromatic JNDs were between 1-3, with none exceeding 5.7 (Fig. 3). Like the koel, four host species had eggs that were significantly more discriminable in luminance from Australasian Figbird and Little Friarbird eggs than from the eggs of all the other species (Kruskal-Wallis:  $\chi^2_{27} = 1070.77$ ,  $P < 0.001$ ; Fig. 3). All average chromatic and achromatic JNDs comparing fresh host eggs were between 1-3, indicating these three hosts have eggs that are just distinguishable in colour and luminance in good lighting conditions (Fig. 4). Fresh Red Wattlebird and Noisy Friarbird eggs were significantly more similar in colour than they were to Magpie-lark egg colour (Kruskal-Wallis:  $\chi^2_5 = 65.22$ ,  $P < 0.001$ ; Fig. 4), but there were no significant differences in luminance between any of the fresh host egg comparisons (Kruskal-Wallis:  $\chi^2_5 = 17.22$ ,  $P = 0.004$ ; Dunn test:  $P > 0.05$  for all tests).

The Magpie-lark and Olive-backed Oriole egg patterns appear to be distinctive when compared to the other host species. The Australasian Figbird, Noisy Friarbird and Red Wattlebird all had egg patterns that were significantly more different from these two hosts than from the egg patterns of the other species (Kruskal-Wallis:  $\chi^2_{27} = 1009.93$ ,  $P < 0.001$ ; Fig. 5). Little Friarbird egg pattern was significantly more different from these two hosts than from the egg patterns of three other host species and the Blue-faced Honeyeater's egg pattern was significantly more similar to Red Wattlebird egg pattern than to the Magpie-lark and Olive-backed Oriole (Fig. 5).

### *Egg Size*

Koel eggs were significantly larger in volume than all of their host eggs and most host eggs were significantly different in size to other species' eggs, with Olive-backed Orioles having the largest host eggs and Little Friarbirds having the smallest (ANOVA:  $F_{7, 550} = 284.80$ ,  $P < 0.001$ ; Fig. 6). Host species was the only significant predictor of koel egg size (LM:  $F_{5, 71} = 3.05$ , Adjusted  $R^2 = 0.12$ ,  $P = 0.02$ ). Koel eggs in Little Friarbird clutches were significantly smaller than koel eggs in clutches of every other host species, except for those in Red Wattlebird clutches (Fig. 7). Koel eggs in Red Wattlebird clutches were significantly smaller than koel eggs in Australasian Figbird clutches (Fig. 7). Similarly, when we substituted the variable "host egg size" for "host species" in the model, host egg size was the only significant predictor of koel egg size (LM:  $F_{1,73} = 7.30$ , Adjusted  $R^2 = 0.08$ ,  $P = 0.01$ ), with koel eggs increasing in volume as host eggs increased in volume ( $t$ -test:  $t = 2.70$ ,  $SE = 0.07$ ,  $P = 0.01$ ).

## DISCUSSION

We found no clear evidence that koels have evolved host-specific races, as koel egg colour and pattern did not separate into distinct groups based on host species. Koel eggs were consistently similar to the eggs of one primary host, the Noisy Friarbird, in all colour and pattern analyses. Koel eggs also showed strong similarities to the eggs of a minor host, the Blue-faced Honeyeater and to the newest host, the Red Wattlebird. However, the egg colours of these three hosts were themselves indistinguishable from one another through the eyes of a bird. Koel eggs did not show as consistent similarities to the eggs of the other hosts across all egg traits, although there were several points of overlap. For instance, Olive-backed Oriole eggs were indistinguishable from koel eggs in colour in the JND analysis, but showed significant differences in egg pattern when compared to koel eggs and the majority of the other host species.



The average chromatic JNDs between the koel and each of its hosts for both the museum and fresh egg datasets were below two and the majority of average achromatic JNDs were below three. Indeed, all of the chromatic JNDs and most of the achromatic JNDs comparing the colour and luminance of the different host species' eggs were between 0-3. These results indicate that the majority of species in this study have similar egg colours which are either indistinguishable or just distinguishable in good lighting conditions and many of the species have similar egg luminance. Additionally, we found all the species, apart from the Magpie-lark and Olive-backed Oriole, have similar egg patterns. These results suggest that koels have evolved a single egg type that mimics that of a primary host, the Noisy Friarbird, which has allowed them to exploit other hosts with similar egg morphology.

The similarity in colour, luminance and pattern between koel eggs and Noisy Friarbird eggs is not surprising, because Noisy Friarbirds are a long-standing, primary host with good egg discrimination abilities (Abernathy 2017). By contrast, the similarity in these attributes between koels and Red Wattlebirds was unexpected because Red Wattlebirds rarely reject foreign eggs (Abernathy 2017). However, this similarity is likely to be due to phylogeny rather than selection for egg mimicry. Red Wattlebirds are closely related to Noisy Friarbirds and they shared similar egg colour, luminance and pattern in all analyses. This pre-existing close match between koel and Red Wattlebird eggs may constrain the evolution of egg rejection in Red Wattlebirds, potentially leading to a high likelihood of recognition errors (mistakenly rejecting their own egg) (Lotem et al. 1995; Marchetti 2000; Stokke et al. 2016), and favouring acceptance of koel eggs when parasitism rates are low (Davies et al. 1996). Instead, Red Wattlebirds may rely on other generalised defences including nesting before the koel's breeding season, abandoning nests that are disturbed by the koel or that contain koel eggs laid before the host's laying period (Abernathy and Langmore 2017) and mobbing koels to prevent them from accessing the nest (Abernathy and Langmore 2016; Abernathy 2017).

Koel eggs were also very similar to Blue-faced Honeyeater eggs in colour and luminance and to Olive-backed Oriole eggs in colour, both minor hosts. While Olive-backed Orioles have been shown to eject odd model eggs from their nest (Abernathy, unpubl. data:  $N = 8$  ejections), the egg rejection ability of Blue-faced Honeyeaters is unknown. Olive-backed Oriole egg colour and Blue-faced Honeyeater egg colour and luminance was also extremely similar to that of the Noisy Friarbird, so in this case, it is unclear whether there has been independent selection for egg mimicry of these minor hosts, or whether this is simply a by-product of selection for mimicry of Noisy Friarbird eggs.

Our JND analyses indicate that the majority of hosts may have difficulty distinguishing koel eggs based on colour, luminance or pattern, which might explain why there were differences in koel egg size based on host species. If hosts can recognise and reject koel eggs based on their larger size, this would select for koel eggs that better match the sizes of their host eggs (Davies and Brooke 1988). Several previous studies have shown that hosts nesting in cavities or dome nests with poor lighting conditions rely on egg size to recognise parasitic eggs (Mason and Rothstein 1986; Marchetti 2000; Langmore et al. 2003). Similarly, egg size could be an important cue for the hosts in our study if visual cues are not reliable. Another possibility is that koel eggs have become more closely matched to their host egg sizes to allow for more efficient incubation of their eggs (Davies 2000). Whether koels have evolved host-specific races in egg size is still inconclusive, as the majority of koel eggs laid in different host nests were significantly similar in size (Fig. 7). Indeed, if hosts tend to reject eggs that are much larger than their own eggs, this could have biased our results because only koel eggs that best matched the sizes of their hosts' eggs would have been found by collectors. Further work, including egg rejection experiments using model eggs of different

sizes and molecular genetic analyses of koels reared by different hosts is needed to resolve this issue.

### *Conclusions*

Koels do not appear to have evolved host-specific egg colours or patterns, suggesting that they do not have host-specific races. Further, unlike the “Jack-of-all-trades” strategy described by Feeney et al. (2014), koel egg appearance was not intermediate between that of its hosts’ eggs, as it was significantly more similar in colour and luminance to one of its traditional major hosts than to two other major host species (Fig. 3). Instead, selection for the evolution of host-specific races may be weak in the koel because the majority of its favoured host species have very similar egg morphologies. Thus, evolving egg mimicry of just one of these hosts, the Noisy Friarbird, may have resulted in a concomitant resemblance between the eggs of koels and several other host species even though there may have been little or no selection for mimicry from these species. This seems to be the case for the Red Wattlebird, which is a known new host that only came in contact with the koel since the 1930s and exhibits very little egg rejection (Abernathy 2017; Abernathy and Langmore 2017), yet its eggs are a close match to the koel’s. The only evidence suggesting that koels have host-specific races was that koel eggs laid in the nests of two smaller hosts were smaller on average than koel eggs laid in the nests of some of the larger hosts. However, this could be the outcome of rejection of larger eggs by the smaller hosts and requires further investigation. This study suggests that successful exploitation of new host species may be facilitated by a similarity in egg morphology between the new host and existing hosts, which could slow down the evolution of host defences if the host is naïve.

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TABLES AND FIGURE LEGENDS

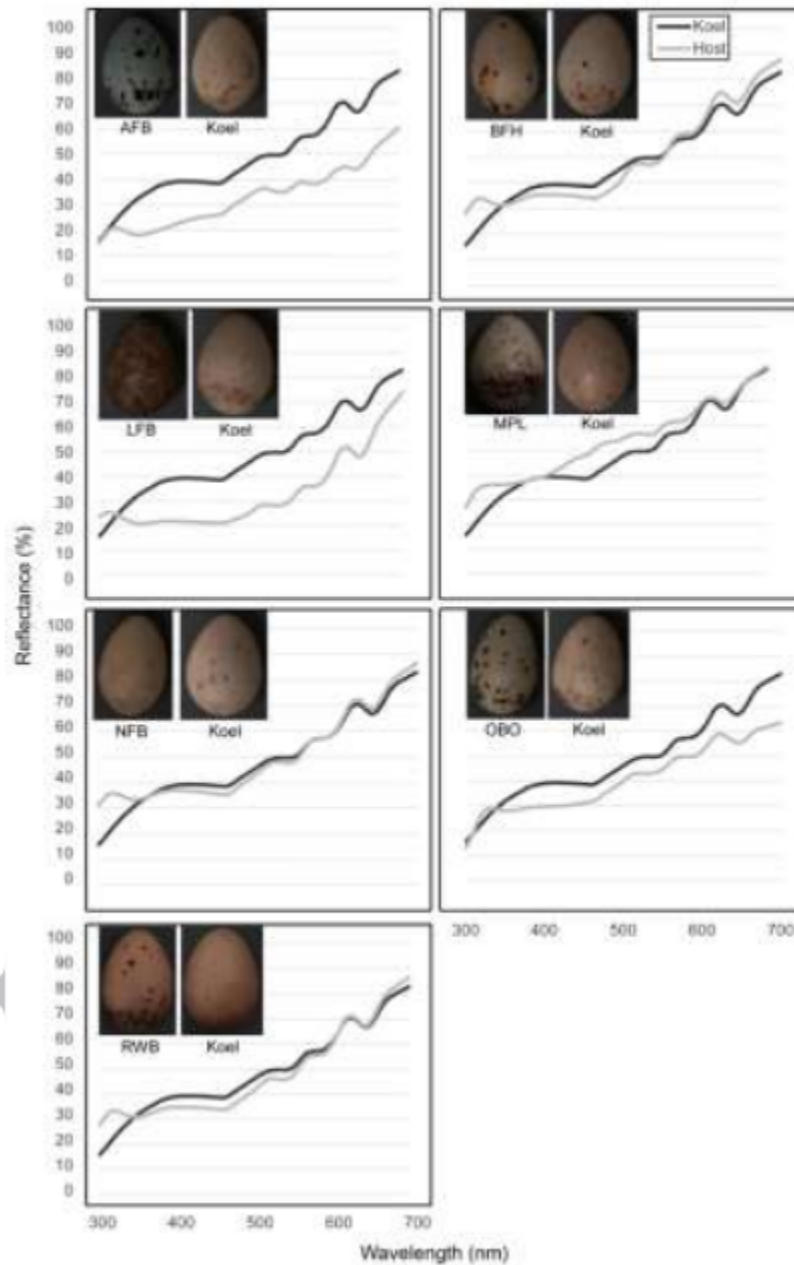
**Table 1.** Sample sizes of host clutches and koel eggs for each state used in three analyses. “Unknown” = state where egg was collected was unknown, “QLD” = Queensland, “NSW” = New South Wales, “ACT” = Australian Capital Territory, “VIC” = Victoria, “SA” = South Australia, “WA” = Western Australia, “AFB” = Australasian Figbird, “BFH” = Blue-faced Honeyeater, “LFB” = Little Friarbird, “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “OBO” = Olive-backed Oriole and “RWB” = Red Red Wattlebird, “Koel” = Pacific Koel. For each state, “U” = the number of unparasitised clutches and “P” = the number of parasitised clutches that were measured. Museum clutches were from the Australian National Wildlife Collection, CSIRO, ACT. Fresh eggs were from Western Sydney, NSW and Canberra, ACT. Fresh koel eggs were only found in Red Wattlebird nests. For the museum egg colour analysis, seven koel eggs and seven Red Wattlebird clutches were collected fresh from Sydney, then blown prior to measuring.

	Host	Year Range	Un-known	QLD	NSW	ACT	VIC	SA	WA	Total
Fresh Egg Colour	MPL	2013-2015			U:8	U:7				15
	NFB	2013-2015			U:2	U:11				13
	RWB	2013-2015			U:20 P:8	U:17				45
	Koel	2014-2015			19					19
Museum Egg Colour	AFB	1898-1990		U:40 P:8	U:3 P:2					53
	BFH	1899-1999	U:2	U:14 P:6	U:10					32
	LFB	1884-2002		U:27 P:10	U:10				U:1	48
	MPL	1897-2004		U:14 P:6	U:21 P:7	U:1	U:7	U:16		72
	NFB	1898-1999		U:17 P:4	U:14 P:10	U:2	U:3			50
	OBO	1896-1999	U:2	U:22 P:4	U:18	U:1	U:3			50
	RWB	1889-2014		U:5	U:18 P:2	U:7	U:13	U:27	U:4	76
	Koel	1901-2014		46	30					76
Egg Pattern	AFB	1898-2014		U:40 P:8	U:4 P:2					54
	BFH	1899-1999	U:2	U:14 P:6	U:10					32
	LFB	1884-2002		U:27 P:10	U:10				U:1	48
	MPL	1897-2015		U:14 P:6	U:29 P:7	U:8	U:7	U:16		87
	NFB	1898-2015		U:18 P:4	U:17 P:10	U:13	U:3			65

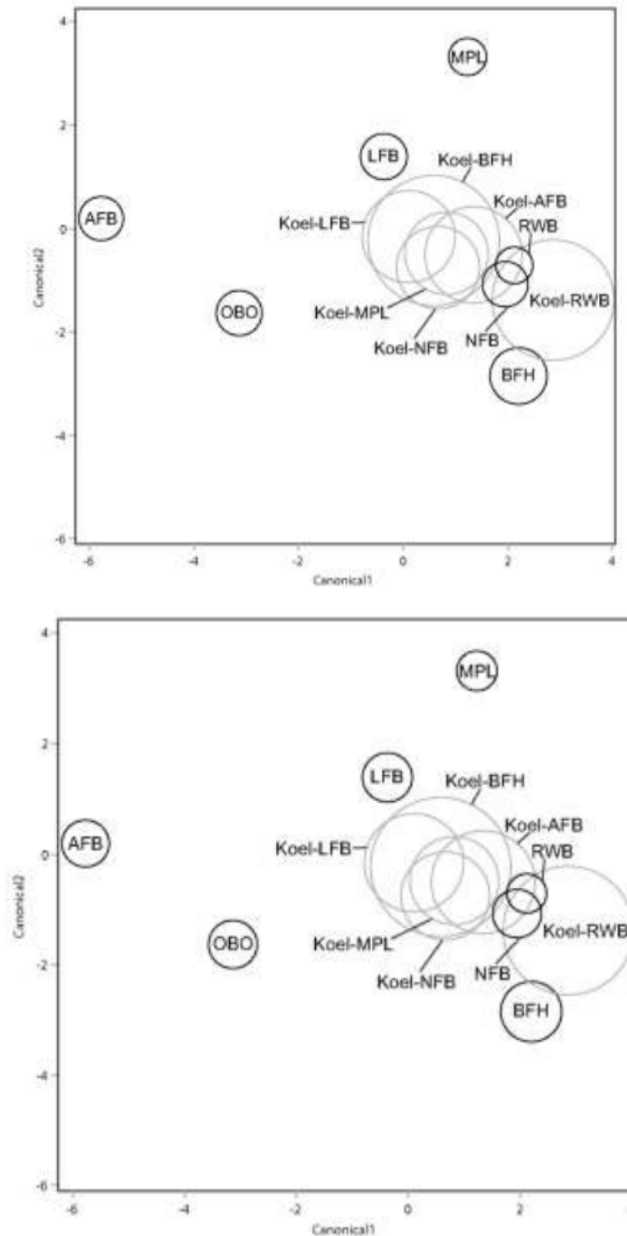
OBO	1896-1999	U:2	U:22 P:4	U:18	U:1	U:3			50
RWB	1889-2015		U:5	U:33 P:7	U:27	U:13	U:27	U:4	116
Koel	1901-2014		46	38					84

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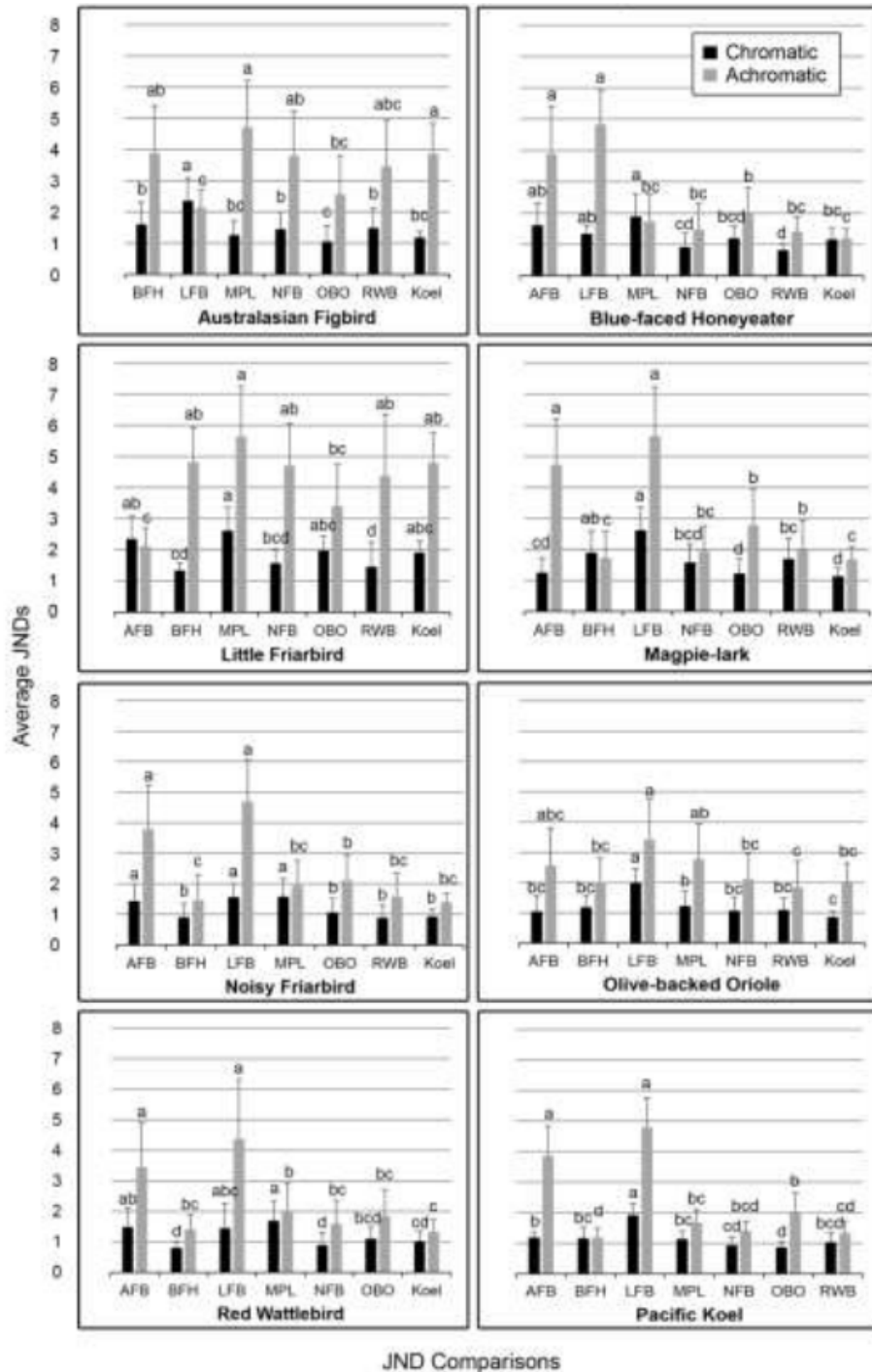
**Figure 1.** Average spectral reflectance and representative photos of Pacific Koel (“Koel”) eggs (black line) compared to eggs of seven host species (grey line, “AFB” = Australasian Figbird, “BFH” = Blue-faced Honeyeater, “LFB” = Little Friarbird, “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “OBO” = Olive-backed Oriole and “RWB” = Red Red Wattlebird). Eggs measured were used in the museum egg analysis (see Table 1 for sample sizes). Egg photos were taken by V.E. Abernathy from 2013-15.



**Figure 2.** Canonical plots from discriminant function analyses separating (a) egg colour and (b) egg pattern of seven koel host species (black rings; “AFB” = Australasian Figbird, “BFH” = Blue-faced Honeyeater, “LFB” = Little Friarbird, “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “OBO” = Olive-backed Oriole and “RWB” = Red Red Wattlebird) and six groups of koel eggs (grey rings) grouped by the host species clutch in which they were found (ex: “Koel-AFB” = koel eggs from Australasian Figbird clutches). Each multivariate mean is labelled with a circle, the size of which represents the 95% confidence limit for the mean. Groups with overlapping circles are statistically similar.

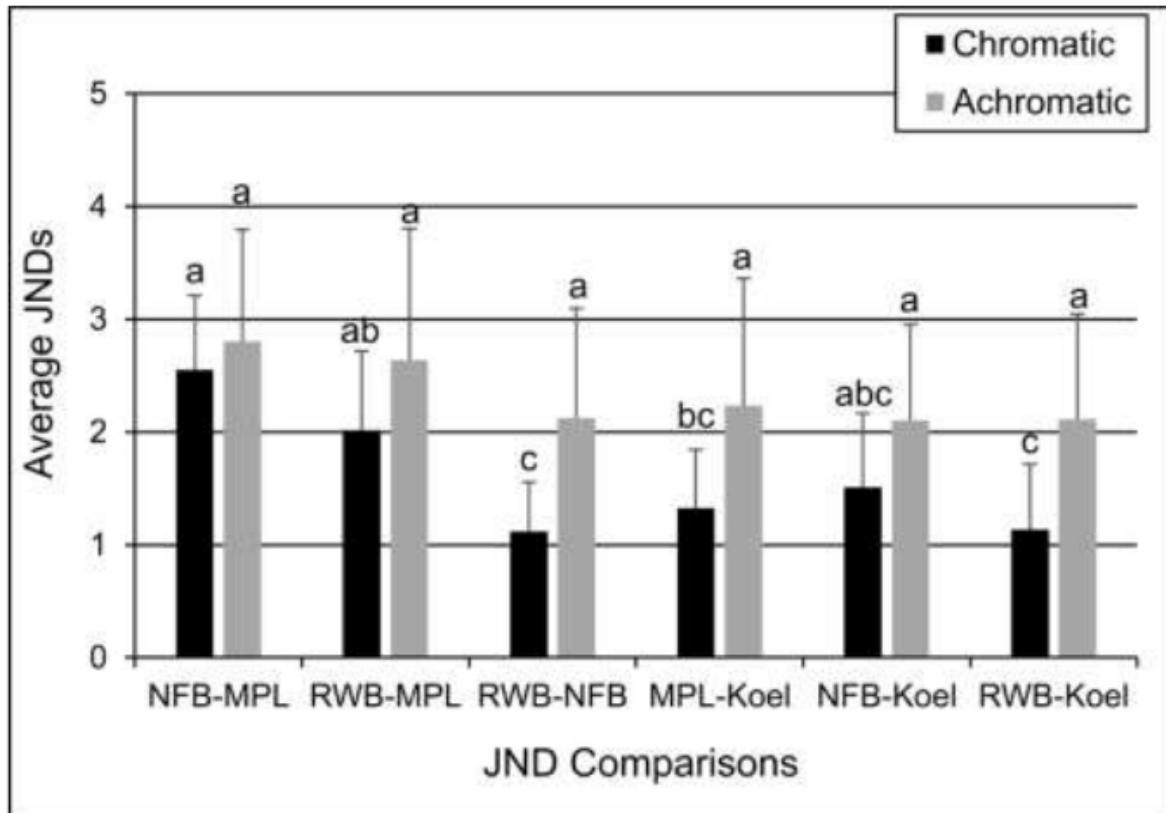


**Figure 3.** Museum egg colour analysis: average chromatic (colour) and achromatic (luminance) JNDs  $\pm$  standard deviations between eggs of the koel and seven of its host species. Each graph shows a single species compared to every other species. Letters above columns indicate significant differences between groups (Dunn post-hoc tests,  $P < 0.05$ ). The chromatic and achromatic analyses were performed separately. “AFB” = Australasian Figbird, “BFH” = Blue-faced Honeyeater, “LFB” = Little Friarbird, “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “OBO” = Olive-backed Oriole, “RWB” = Red Red Wattlebird and “Koel” = Pacific Koel (see Table 1 for sample sizes).

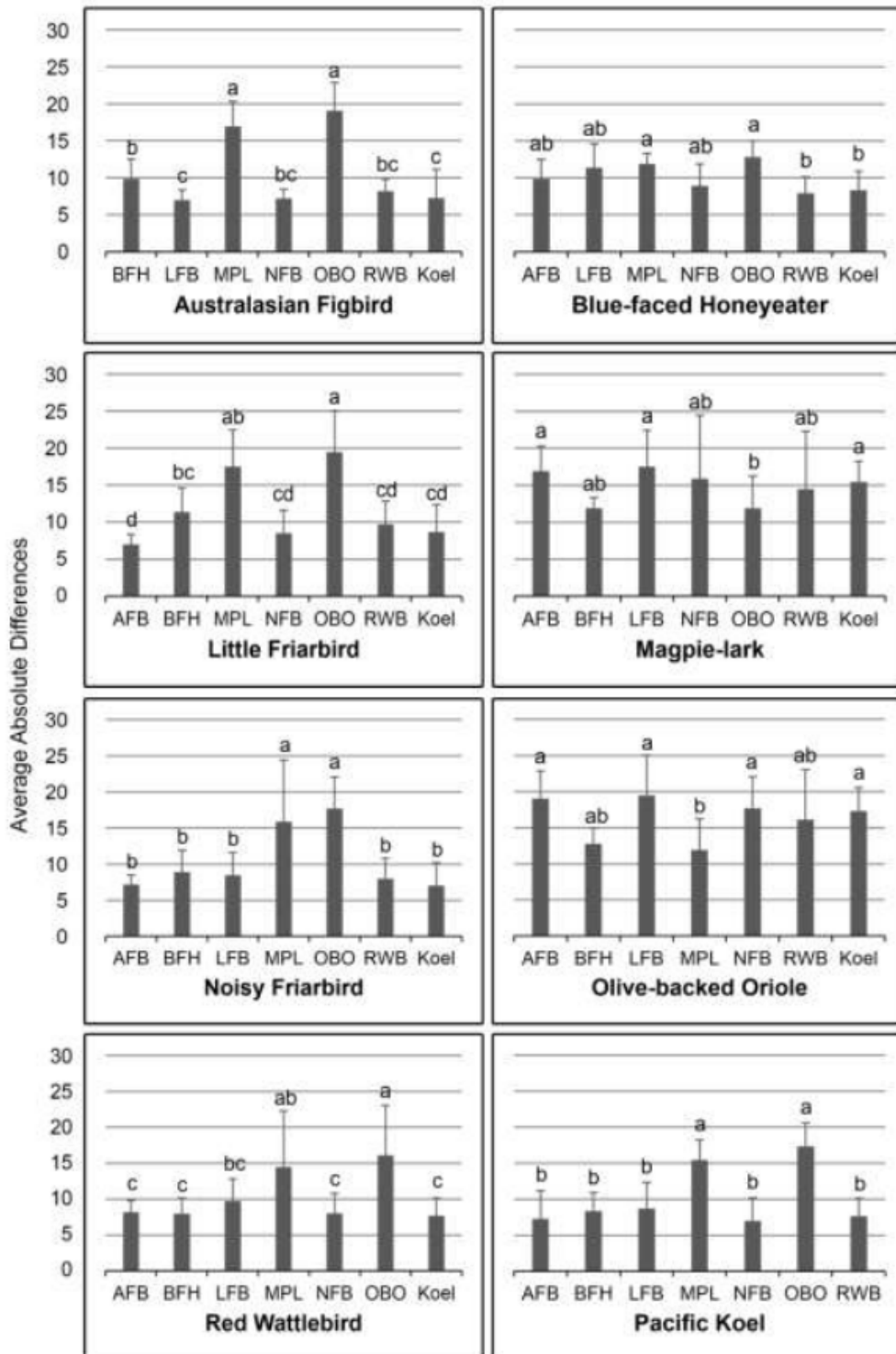




**Figure 4.** Fresh egg colour analysis: average chromatic (colour) and achromatic (luminance) JNDs  $\pm$  standard deviations between eggs of three koel host species and koel eggs and between eggs of each host species. Letters above columns indicate significant differences between groups (Dunn post-hoc tests,  $P < 0.05$ ). The chromatic and achromatic analyses were performed separately. “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “RWB” = Red Red Wattlebird and “Koel” = Pacific Koel (see Table 1 for sample sizes).

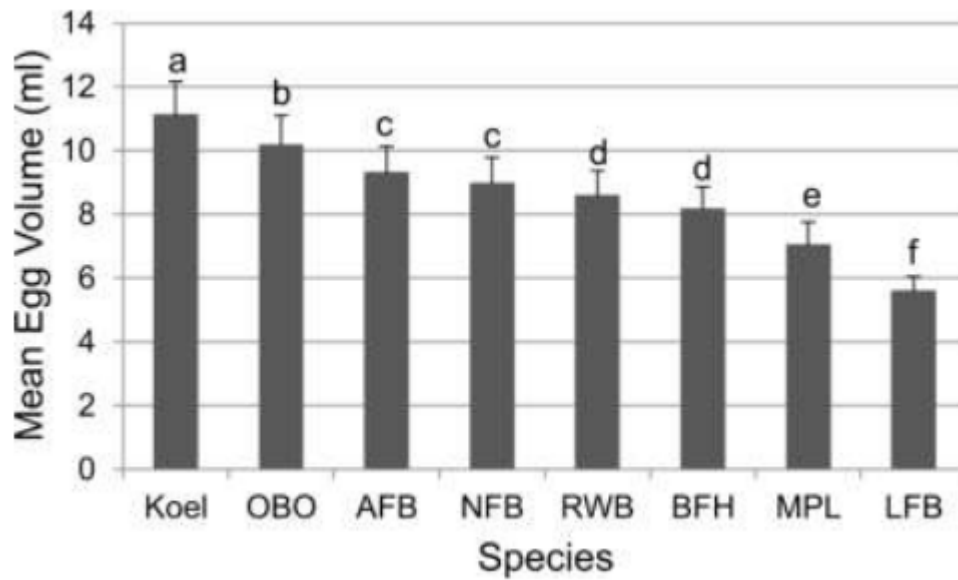


**Figure 5.** Egg pattern analysis: average absolute differences in egg pattern energy  $\pm$  standard deviations between eggs of the koel and seven of its host species. Each graph shows a single species compared to every other species. Letters above columns indicate significant differences between groups (Dunn post-hoc tests,  $P < 0.05$ ). “AFB” = Australasian Figbird, “BFH” = Blue-faced Honeyeater, “LFB” = Little Friarbird, “MPL” = Magpie-lark, “NFB” = Noisy Friarbird, “OBO” = Olive-backed Oriole, “RWB” = Red Red Wattlebird and “Koel” = Pacific Koel (see Table 1 for sample sizes).



Egg Pattern Comparisons

**Figure 6.** Average egg volumes (ml)  $\pm$  standard deviations for the Pacific Koel and seven of its host species. Letters above each column indicate significant differences between species (Tukey HSD,  $P < 0.05$ ). “Koel” = Pacific Koel,  $N = 88$ ; “OBO” = Olive-backed Oriole,  $N = 50$ ; “AFB” = Australasian Figbird,  $N = 59$ ; “NFB” = Noisy Friarbird,  $N = 68$ ; “RWB” = Red Red Wattlebird,  $N = 123$ ; “BFH” = Blue-faced Honeyeater,  $N = 32$ ; “MPL” = Magpie-lark,  $N = 90$ ; “LFB” = Little Friarbird,  $N = 48$ .



**Figure 7.** Average egg volumes (ml)  $\pm$  standard deviations for Pacific Koel eggs in clutches of six host species. Letters above each column indicate significant differences between koel eggs in clutches of different host species (LM: *t*-test,  $P < 0.05$ ). “AFB” = Australasian Figbird,  $N = 11$ ; “NFB” = Noisy Friarbird,  $N = 13$ ; “RWB” = Red Red Wattlebird,  $N = 20$ ; “BFH” = Blue-faced Honeyeater,  $N = 6$ ; “MPL” = Magpie-lark,  $N = 15$ ; “LFB” = Little Friarbird,  $N = 12$ .

