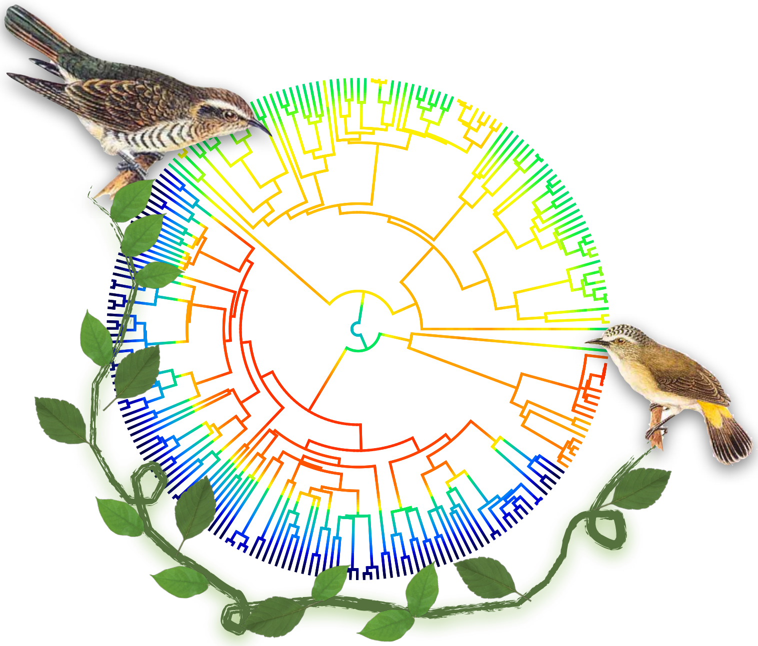
Macroevolutionary outcomes of coevolution between avian brood parasites and their hosts



Iliana Medina Guzmán
Supervisor: Dr. Naomi Langmore

Australian
National
University

A thesis submitted for the degree of Doctor of Philosophy
The Australian National University
May 2016

DECLARATION

The research presented in this thesis is my own original work except where due reference is given in the text. All of the chapters are coauthored. Unless otherwise indicated, the authorship order indicates the intellectual input and workload. No part of this thesis has been submitted for any previous degree.

Iliana Medina Guzmán

May 2016

ACKNOWLEDGEMENTS

First, I would like to thank my supervisor Naomi Langmore for her huge support during my entire PhD, for being always so enthusiast and encouraging every time we discussed new ideas or recent results, for always showing great commitment with her role as my supervisor and mentor and for being a constant source of inspiration and motivation.

I would also like to thank my collaborators; Jolyon Troscianko, Will Feeney, Martin Stevens, Rob Lanfear, Michelle Hall, Leo Joseph, Robert Palmer and my supervisory panel; Rob Heinsohn and Hanna Kokko for very insightful discussions. I am also very thankful to my field assistants Michael Warren, T.J. Keyes and Hazel Speed, and to Kristal Cain, Thomas Wallenius, Constanza León, Thomas Merkling and Wendy Dimond for all the help finding nests. I would like to give special thanks to Will Feeney who has been extremely helpful during all the stages of my PhD, and to Hanna Kokko, for being so enthusiast with the project and always willing to explain and discuss.

Finally, I would like to thank all the wonderful people that made EEG a great place to be. I'd like to thank my very good friends Coni, Regi, Carlos, Damien, Marta, Joshie, Bo, Dave K, Ana S., Dani, Moos, Hannah and the metrognomes, Dan S., Anna H., Dan H., Amanda and everyone at the PhD office and all the EEG staff and professors. I'd also like to thank Tomás Wallenius, for making every moment happy and exciting. And last, but not least, I would like to thank my wonderful friends Ana B., Natalia and Ceci, and my family, Rocío, Alfonso and Camila, because they are my inspiration, and every task is much easier with their support and energy. Son mi inspiración!

ABSTRACT

Almost one hundred bird species in the world are known to be obligate interspecific brood parasites. These lay their eggs in the nests of other species, their hosts, which take care of a usually larger parasitic chick. Brood parasitism constitutes one of the best examples of coevolution in the animal kingdom. This strategy is usually costly to the host, and has led to the evolution of a suite of adaptations in hosts, in order to defend themselves against parasitism, and in parasites, in order to effectively parasitize their hosts. In this thesis I explore the effects of brood parasitism on macro-evolutionary patterns in both hosts and parasites.

In the first six chapters of my thesis I explore how defences evolve in hosts. First I present a literature review about the evolution of egg acceptance and tolerance mechanisms in hosts of brood parasites, in which I discuss how other co-evolutionary interactions, such as those between plants and herbivores, may be informative for understanding brood parasitic systems. In the second chapter I perform a large-scale comparative analysis on the evolution of clutch size as a tolerance mechanism in hosts. This chapter also incorporates a mathematical model and a field experiment on the Horsfield's bronze-cuckoo *Chalcites basalis*. In the third chapter I investigate why one type of defence, egg rejection, evolves in some host species and not in others. In the fourth chapter I present a comparative analysis which tests the idea that the benefits of group defence against brood parasites has led to the evolution of cooperative breeding in hosts. For the fifth and sixth chapters, I describe field experiments to test the evolution of defences in the yellow-rumped thornbill (*Acanthiza chrysorrhoa*), the main host of the shining bronze-cuckoo (*Chalcites lucidus*) in Australia. My main aim is to understand which types of defences have evolved in this major host. I also perform field experiments to understand which factors constrain the evolution of defences in this species.

In the second part of my thesis I study how brood parasitism can be associated with the evolution of diversity in both hosts and parasites, especially in traits that are likely to be under selective pressures, such as the egg phenotype. In chapter 7 I study how egg phenotype has evolved to be more diverse within and among species that are hosts of brood parasites. In chapter 8 I explore whether a brood parasitic breeding strategy promotes the generation of new species and phenotypic diversity. Specifically, I test whether brood parasitic lineages have faster rates of speciation and phenotypic evolution.

Finally, in chapter 9, I discuss how together, these chapters offer a broad evolutionary landscape that demonstrate the diverse impacts of brood parasitism as a co-evolutionary interaction. I provide evidence that brood parasitism, besides driving the evolution of defenses, is linked to trait diversity, and may be an important force behind the evolution of clutch size, cooperative breeding, egg pattern, egg size and plumage diversity.

THESIS OUTLINE

The following chapters compose this thesis:

1. Evolution of acceptance and tolerance in hosts of avian brood parasites.
   Iliana Medina & Naomi E. Langmore.
   Biological Reviews (2015) 90 (3): 1-6

2. The evolution of clutch size in hosts of avian brood parasites.
   Iliana Medina, Naomi E. Langmore, Michelle Hall, Raoul Mulder, Rob Lanfear & Hanna Kokko.
   Resubmitted with revisions to *Evolution*

3. The costs of avian brood parasitism explain variation in egg rejection behaviour in hosts.
   Iliana Medina & Naomi E. Langmore.
   Biology Letters (2015) 11 (7): 1-3

4. Brood parasitism and the evolution of cooperative breeding in birds.
   William E. Feeney, **Iliana Medina**, Marius Somveille, Robert Heinsohn, Michelle L. Hall, Raoul A. Mulder, John A. Stein, Rebecca M. Kilner and Naomi E. Langmore.
   Science (2013) 342: 1506-1508

5. Batten down the thatches: Front-line defences in an apparently defenceless cuckoo host.
   Iliana Medina & Naomi E. Langmore.
   Animal Behaviour 112:195-201.

6. Nest illumination and the evolution of egg rejection in hosts of brood parasites.
   Iliana Medina & Naomi E. Langmore.

7. Brood parasitism is linked to egg pattern diversity within and among species of Australian passerines.
   Iliana Medina, Jolyon Troscianko, Martin Stevens & Naomi E. Langmore.
   The American Naturalist 187(3): 351-362.

8. Coevolution is linked to phenotypic diversity but not speciation in avian brood parasites.
   Iliana Medina & Naomi E. Langmore.
   Proceedings of the Royal Society 282: 1-8.

9. Synthesis and general conclusions.

TABLE OF CONTENTS

INTRODUCTION

Co-evolution is arguably one of the most fascinating processes in nature. Even before Darwin, naturalists were amazed by the finely tuned adaptations of pollinators to the flowers they exploit, and by the strategies that flowers employ in order to be pollinated. In animals, avian brood parasitism is one of the best-known examples of coevolution. Obligate interspecific brood parasites lay their eggs in the nests of their hosts, which then take care of a usually larger parasitic chick (Davies 2000). Almost 100 species of birds are brood parasites, and more than 500 bird species are their hosts (Friedmann and Kiff 1985; Brooker and Brooker 1989; Soler 2014). In Australia, 32% of passerine species are major hosts of brood parasites (Brooker and Brooker 1989).

Brood parasitism has evolved independently seven times in the avian phylogeny (Figure 1, Jetz et al. 2012). The largest and best-known lineage of brood parasites is the cuckoos (Cuculidae, Subfamily: Cuculinae, 52 spp.), which have mainly an old-world distribution (Payne and Payne 1998). In Africa, two other families of brood parasites have evolved; the honeyguides (Indicatoridae, 20 spp.) and the Vidua finches (Viduidae, 17 spp.). In the Americas, there are parasitic cowbirds (Icteridae, 5 spp.) and a parasitic duck (*Heteronetta atricapilla*). Although all these lineages are brood parasitic, they employ a diversity of strategies to exploit their hosts. For instance, many parasitic cuckoos and honeyguides kill the progeny of the host, by evicting the host eggs or chicks from the nest (cuckoos) or by stabbing their foster siblings with a hook in their bills (honeyguides, Spottiswoode and Koorevaar 2011). Conversely, in Vidua finches, cowbirds and some cuckoos, the progeny of the host may be raised alongside the parasitic chick (Sorenson et al. 2003). Finally, the brood parasitic black-headed duck becomes independent soon after hatching and appears to impose little or no costs on its hosts (Lyon & Eadie 2004). Thus the costliness, or 'virulence', of brood parasites varies widely, depending on the strategies they employ to exploit their hosts (Kilner 2005). It is highly likely that such variation in virulence has important implications in the coevolutionary interactions of hosts as parasites, given that selection for defenses might be weaker if hosts do not lose all their progeny.

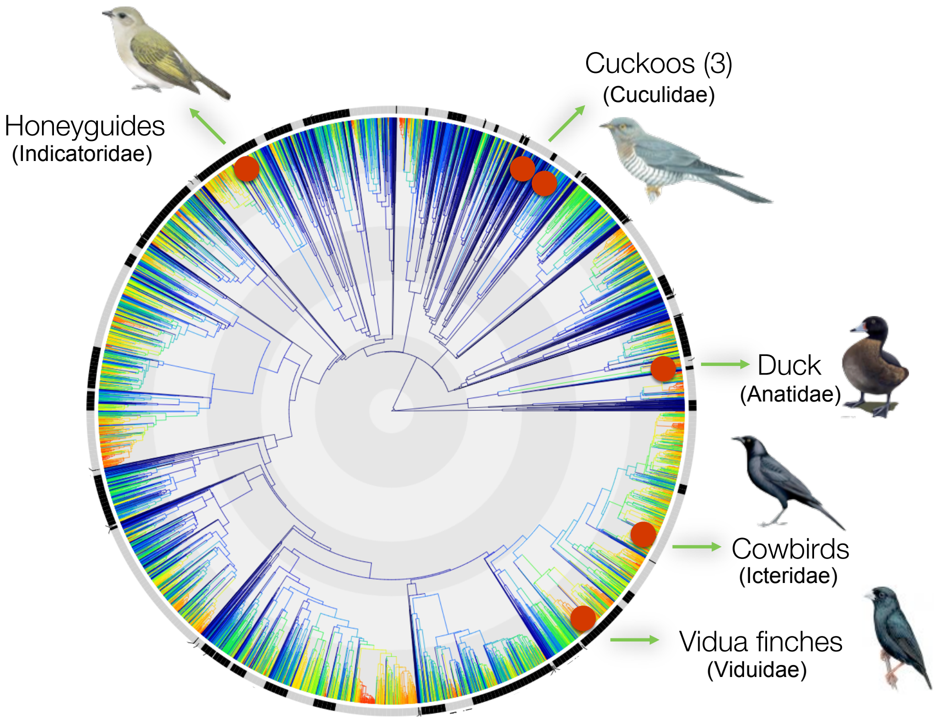


Figure 1. Phylogeny of birds of the world (taken from Jetz et al. 2012). Arrows show phylogenetic positioning of brood parasitic lineages.

Avian brood parasitism is a very widespread strategy in nature. Brood parasites are phylogenetically scattered, each parasitic species may exploit up to 170 different species of hosts (Friedmann and Kiff 1985), and rates of parasitism can be very high within host populations (eg. 63% of host nests, Gloag et al. 2014). Thus brood parasitism is a strategy that affects hundreds of bird species, on all continents in the world. The main aim of this thesis is to explore the macro-evolutionary patterns that such a strategy might generate, in both hosts and parasites.

Hosts usually experience high costs as a result of being parasitized. One of the best studied types of defence is egg rejection (Soler 2014), and many (but not all) host species are able to reject parasitic eggs based on cues such as colour or size (Spottiswoode and Stevens 2010). Rejection of parasitic eggs is absent in more than 30%

of major hosts (Soler 2014), these species might be using other strategies. Hosts of brood parasites may employ other types of defences such as tolerance, where hosts reduce the costs of being parasitized by modifying life-history traits (Svensson & Raberg 2010). For my first chapter (Ch. 1) I present a literature review that explores the circumstances under which tolerance of brood parasites can evolve, and the possible evolutionary outcomes of this interaction. We base our predictions on published literature on plant-herbivore and host-parasite interactions. In Chapter 2, I present an empirical test of tolerance through clutch size reduction. With the help of collaborators, we test whether clutch size reduction has evolved as a strategy to reduce the costs in hosts of brood parasites in America, Africa and Australia.

In my third chapter (Ch. 3) I present information on almost 200 published egg rejection experiments and perform comparative analyses to understand the conditions under which egg rejection is likely to evolve. Moreover, some types of defences such as mobbing may have triggered the evolution of further defensive behaviours. Chapter 4 explores the hypothesis that cooperative breeding has evolved in conjunction with being a host a brood parasite; on one side, hosts may benefit from breeding in larger groups if this increases the vigilance and nest protection from parasites. On the other side, brood parasites may benefit from laying their eggs in nests where not only parents but also helpers aid feeding their chick.

In chapters five and six I use the study system of the shining bronze-cuckoo (*Chalcites lucidus*) and it's main host, the yellow-rumped thornbill (*Acanthiza chrysorrhoa*), to understand at a finer scale how different types of defences interact within a population. I perform four different experiments in a population in Canberra, to explore how multiple defences can be present in a population and why the establishment of some defences might be constrained.

Besides driving the evolution of defence strategies, brood parasitism can also be an important factor in promoting diversity. Co-evolution has been linked to species diversity in pollinator-plant interactions and in host-plant interactions (Hodges and Arnold 1995; Cook and Rasplus 2003; Janz et al. 2006). In brood parasites, it has been suggested that a brood parasitic breeding strategy is linked to increased rates of speciation (Krüger et al. 2009). In chapters 7 and 8 I explore whether brood parasitism is linked with higher diversity in the egg appearance of hosts (Ch. 7) and in phenotypic traits and speciation in brood parasites (Ch. 8).

In my last chapter I present a synthesis of the main aim of this thesis and main findings. Overall, the intention of this thesis is to understand the influence of brood parasitism at a large scale, within hosts and within parasites. The development of novel phylogenies (Jetz et al. 2012) and phylogenetic methods (Harmon et al. 2008; Rabosky 2014) has allowed me to explore in depth the importance of brood parasitism in a macro-evolutionary context.

References

Brooker, M. G. and L. C. Brooker. 1989. Cuckoo hosts in Australia. Australian Zoological Reviews 2:1-67.

Cook, J. M. and J.-Y. Rasplus. 2003. Mutualists with attitude: coevolving fig wasps and figs. Trends in Ecology & Evolution 18:241-248.

Davies, N. B. 2000. Cuckoos, cowbirds and other cheats. T. & A.D. Poyser, London.

Friedmann, H. and L. Kiff. 1985. The parasitic cowbirds and their hosts. Western foundation of vertebrate zoology 2:225-302.

Harmon, L. J., J. T. Weir, C. D. Brock, and R. E. Glor. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129-131.

Hodges, S. A. and M. L. Arnold. 1995. Spurring plant diversification: Are floral nectar spurs a key innovation? Proceedings of the Royal Society 262:343-348.

Janz, N., S. Nylin, and N. Wahlberg. 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. BMC Evolutionary biology 6:1-10.

Jetz, W., G. Thomas, A. Hartmann, and O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444-448.

Kilner, R. M. 2005. The evolution of virulence in brood parasites. Ornithological Science 4:55-64.

Krüger, O., M. D. Sorenson, and N. B. Davies. 2009. Does coevolution promote species richness in parasitic cuckoos? Proceedings of the Royal Society B: Biological Sciences 276:3871-3879.

Payne, R. B. and L. L. Payne. 1998. Parasitic Birds and their Hosts. Studies in Coevolution. Oxford University Press, Oxford.

Rabosky, D. 2014. Automatic detection of Key innovations, rate shifts, and diversity-dependence on phylognetic trees. Plos ONE 9:e89543.

Soler, M. 2014. Long-term coevolution between avian brood parasites and their hosts. Biological Reviews 89:688-704.

Sorenson, M. D., K. M. Sefc, and R. B. Payne. 2003. Speciation by host switch in brood parasitic indigobirds. Nature 424:928-931.

Spottiswoode, C. N. and J. Koorevaar. 2011. A stab in the dark: chick killing by brood parasitic honeyguides. Biology Letters 10.1098/rsbl.2011.0739.

Spottiswoode, C. N. and M. Stevens. 2010. Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs. Proceedings of the National Academy of Sciences, USA 107:8672-8676.

Chapter 1

The evolution of acceptance and tolerance in hosts of avian brood parasites.

Published in *Biological Reviews* (2015)

BIOLOGICAL REVIEWS
Cambridge Philosophical Society

Biol. Rev. (2015), pp. 000–000.
doi: 10.1111/brv.12181

The evolution of acceptance and tolerance in hosts of avian brood parasites

Iliana Medina* and Naomi E. Langmore

Evolution, Ecology and Genetics, Research School of Biology, The Australian National University, Canberra, Australia

ABSTRACT

Avian brood parasites lay their eggs in the nests of their hosts, which rear the parasite's progeny. The costs of parasitism have selected for the evolution of defence strategies in many host species. Most research has focused on resistance strategies, where hosts minimize the number of successful parasitism events using defences such as mobbing of adult brood parasites or rejection of parasite eggs. However, many hosts do not exhibit resistance. Here we explore why some hosts accept parasite eggs in their nests and how this is related to the virulence of the parasite. We also explore the extent to which acceptance of parasites can be explained by the evolution of tolerance; a strategy in which the host accepts the parasite but adjusts its life history or other traits to minimize the costs of parasitism. We review examples of tolerance in hosts of brood parasites (such as modifications to clutch size and multi-broodedness), and utilize the literature on host–pathogen interactions and plant herbivory to analyse the prevalence of each type of defence (tolerance or resistance) and their evolution. We conclude that (*i*) the interactions between brood parasites and their hosts provide a highly tractable system for studying the evolution of tolerance, (*ii*) studies of host defences against brood parasites should investigate both resistance and tolerance, and (*iii*) tolerance and resistance can lead to contrasting evolutionary scenarios.

Key words: brood parasitism, tolerance, resistance, hosts, egg rejection, strategy blocking, evolutionary equilibrium.

CONTENTS

---

* Address for correspondence (Tel: +61 2 6125 3828; E-mail: iliana.medina@anu.edu.au).

I. INTRODUCTION

Avian interspecific brood parasites exhibit fascinating adaptations to avoid the costs of raising their offspring. Instead of building a nest, they lay their eggs in the nests of other species and abandon their young to the care of the hosts (Davies & Brooke, 1988; Davies, 2000). Brood parasitism can be highly costly for hosts and this antagonistic interaction can select for resistance in the host and counter-adaptations in the parasite (Dawkins & Krebs, 1979; Davies, 2000). Consequently, the interactions between brood parasites and their hosts are often invoked as an example of a coevolutionary arms race (Dawkins & Krebs, 1979; Davies, 1989; Foitzik, Fischer & Heinze, 2003; Lyon & Eadie, 2004). Alternatively, hosts may defend themselves against brood parasites by adjusting their breeding strategy to minimize the fitness costs of parasitism. Unlike resistance, this strategy, termed tolerance, does not have a negative impact on brood parasite fitness, and is unlikely to give rise to a coevolutionary arms race between parasite and host (Svensson & Råberg, 2010; Fornoni, 2011).

Resistance is defined as a response that either prevents or reduces (moderate resistance) the number or extent of enemy attacks (Svensson & Råberg, 2010). Hosts have evolved multiple levels of resistance against avian brood parasites. At the front line of brood parasite–host interactions (prior to egg deposition), hosts can resist parasites through mobbing (Feeney, Welbergen & Langmore, 2012), breeding in larger groups to facilitate nest defence (Canestrari, Marcos & Baglione, 2009; Feeney *et al.*, 2013) or adjusting their breeding phenology to reduce synchrony with the parasite (Brooker & Brooker, 1989). Counter-adaptations of brood parasites at this stage of the arms race include cryptic plumage and behaviour (Feeney *et al.*, 2012), and plumage polymorphisms (Thorogood & Davies, 2012) to prevent detection by hosts, and mimicry of raptor plumage to inhibit aggression from hosts (Welbergen & Davies, 2011).

Once the parasitic egg is laid hosts can deploy other forms of resistance to minimize the costs of parasitism. Hosts of many species recognize foreign eggs and reject them using their bills (Moksnes, Røskaft & Korsnes, 1993; Sealy, 1996; Marchetti, 2000) or feet (De Marsico *et al.*, 2013). Egg rejection is the most intensively studied type of defence, and the best-known examples of counter-adaptations by parasites are in response to this type of resistance. Many brood parasites have evolved eggs that match those of their hosts in appearance (Brooke & Davies, 1988; Kilner, 2006; Stoddard & Stevens, 2010, 2011; Spottiswoode & Stevens, 2011) or thickened eggshells to withstand host attacks (Brooker & Brooker, 1991; Picman & Pribil, 1997), exemplifying how the arms race between hosts and parasites has shaped the phenotypic evolution of eggs (Kilner, 2006).

Finally, even if the parasite egg is not rejected from the nest, hosts may still express defences at the final stage of the nesting cycle. Several host species reject brood parasite chicks soon after hatching (Langmore *et al.*, 2009*a*; Sato *et al.*, 2010; Tokue & Ueda, 2010), at the end of the nestling period (Grim, 2007) or even after fledging (De Mársico, Gantchoff & Reboreda, 2012). Counter-adaptations of brood parasites at this stage include mimicry of host nestling begging calls (Redondo & Reyna, 1988; Langmore *et al.*, 2008; Anderson *et al.*, 2009; De Mársico *et al.*, 2012) and morphology (Langmore *et al.*, 2011; De Mársico *et al.*, 2012).

The evolution of resistance in brood parasite hosts has been the subject of extensive research. There are numerous examples of how the evolution of defences depends on the duration of the interaction with the parasite and the rate of parasitism (Briskie, Sealy & Hobson, 1992; Edvardsen *et al.*, 2001; Moskát, Szentpéteri & Barta, 2002; Aviles *et al.*, 2006). However, it is still unclear why some hosts do not evolve defences (Stokke, Moksnes & Røskaft, 2005). In this review we explore why some hosts accept parasitic eggs, how virulence is related to egg acceptance and how tolerance evolves in brood parasite hosts. Finally, we utilize studies of plant tolerance and host–pathogen interactions to generate predictions about the evolutionary implications of tolerance in a brood parasite–host system. Although these fields often have quite distinct bodies of literature, they are in effect a continuum of different types of parasitism that can be explored within a single evolutionary framework. Brood parasitism offers a particularly interesting model system for testing evolutionary theory, because, compared to endoparasitic systems, it is easier to isolate the costs of parasitism and identify the traits that change in response to parasitism.

II. WHY DO HOSTS SPECIES ACCEPT EGGS?

Soler (2014) showed that 36.7% of commonly exploited host species accepted foreign eggs in their nest (less than 10% of eggs were rejected). This percentage seems high, given the theoretical prediction that where parasitism is costly, hosts should evolve defences (Davies, Brooke & Kacelnik, 1996; Davies, 1999). Several hypotheses have been proposed to explain acceptance of parasite eggs by hosts, which relate to the trade-offs between the relative costs and benefits of resistance (strategy blocking and evolutionary equilibrium) and the time and genetic variation necessary for resistance to evolve (evolutionary lag).

(1) Strategy blocking

Strategy blocking refers to the diminishing returns of evolving later lines of defence if earlier lines of defence are successful (Planqué *et al.*, 2002; Britton, Planqué & Franks, 2007; Grim *et al.*, 2011). For example, lack of egg rejection in Cape bulbuls (*Pycnonotus capensis*) may be partially explained by their fierce defence of nests from Jacobin cuckoos (*Clamator jacobinus*) (Krüger, 2011). Similarly, the lack of egg rejection in hosts of Australian bronze-cuckoos (*Chalcites* spp.) may reflect the greater effectiveness of chick rejection as a defence, leading to relaxation of selection for egg rejection (Langmore & Kilner, 2010).

(2) Evolutionary equilibrium

The evolutionary equilibrium hypothesis states that for some populations it is more costly to evolve defences than to evolve acceptance, so acceptance is more adaptive than resistance (Zahavi, 1979; Petit, 1991; Lotem, Nakamura & Zahavi, 1992). This process differs from the evolution of tolerance in that no change in host traits is required for the evolution of an evolutionary equilibrium, whereas adaptive change in a host trait is required for the evolution of tolerance. Thus these two processes result in acceptance of parasitic young through distinct evolutionary routes. An example of an evolutionary equilibrium is provided by the magpie (*Pica pica*) hosts of the great-spotted cuckoo (*Clamator glandarius*), which suffer higher costs of rejecting cuckoo eggs than accepting them, because the parent cuckoo destroys the nests of those individuals that reject their parasitic eggs (Soler *et al*., 1995; Davies, 1999). Cuckoos benefit from destroying magpie offspring because they induce magpies to re-nest, and magpies are forced to accept the cuckoo eggs because it is less costly to raise a cuckoo chick alongside their own young than to lose all their progeny (Soler *et al*., 1995). This is known as the avian mafia hypothesis (Zahavi, 1979; Soler & Soler, 2000).

Egg acceptance can also be adaptive if the probability of rejection errors is high. For instance, discrimination of foreign eggs might be difficult if the host's nest is dark (Langmore *et al*., 2005; Honza *et al*., 2011) leading to a high risk of recognition errors and mistaken rejection of the host's own eggs. Additional costs in the form of damage to the host's own eggs during egg rejection may also be exacerbated in dark nests. Detection of brood parasites may be even further constrained when the brood parasite evolves cryptic eggs, which are indistinguishable from the nest lining inside a dark nest (Langmore *et al*., 2009*b*; Gloag, Keller & Langmore, 2014). Egg (and potentially chick) rejection may also be constrained by mimicry of host eggs and chicks by the parasite. Davies *et al*. (1996) showed that reed warblers (*Acrocephalus scirpaceus*) do best to accept mimetic parasitic eggs at low probabilities of parasitism, owing to the risk of ejecting one of their own eggs. Recognition errors might be related to the age of the host: it has been shown that more experienced breeders are better able to recognize brood parasite eggs, chicks and adults than are naïve birds (Smith, Arcese & McLean, 1984; Hobson & Sealy, 1989; Lotem *et al*., 1992; Lotem, Nakamura & Zahavi, 1995; Langmore *et al*., 2009*a*; Feeney & Langmore, 2013). Egg acceptance may also persist in a population if nest predation is common enough to dilute the negative effects of parasitism; in the rufous-collared sparrow (*Zonotrichia capensis*) high predation rates reduce the relative benefits of antiparasite defences and help to explain their absence in this species (Carro & Fernandez, 2013). Ecological and physical constraints can increase the costs of rejection behaviour. The prothonotary warbler (*Protonotaria citrea*) accepts brood parasite eggs as a consequence of nest-site limitation, since opportunities for re-nesting are rare (Petit, 1991). The small eastern olivaceous warbler (*Hippolais pallida*) recognizes and pecks 87% of foreign eggs, but due to physical constraints (e.g. egg size and thick shell) the actual rejection rate is much lower (43.5%; Antonov *et al*., 2009).

Egg acceptance may be adaptive if host nests are parasitized by multiple parasites, each of which removes a single egg prior to laying. In these circumstances it may be more beneficial for the host to leave all parasite eggs in the nest, thereby reducing the probability of host egg removal during subsequent parasitism events (the 'dilution effect'; Sato *et al*., 2010). Subsequently, hosts may remove the parasite young after hatching (Sato *et al*., 2010), or rear them alongside their own chicks (Gloag *et al*., 2011).

Finally, egg acceptance may also evolve when there are benefits of having a cuckoo chick inside a nest. Recently it was shown that nestlings of the great-spotted cuckoo (*Clamator glandarious*) decrease the chance of predation in nests of carrion crows (*Corvus corone*) due to the production of a repellent secretion (Canestrari *et al*., 2014). In this particular case cuckoo chicks are much smaller than the host chicks, and the benefits of predator deterrence by the cuckoo chick outweigh the costs of raising it. Similarly, chicks of the parasitic giant cowbird (*Scaphidura oryzivora*) preened host chicks in oropendola's (*Zarhynchus wagleri*) nests, protecting them from skin parasites (Smith, 1968), however this study was never replicated (Davies, 2000).

(3) Evolutionary lag hypothesis

This hypothesis states that a lack of defences can be explained by the fact that there has not been sufficient time or genetic potential for the establishment of defences in the population, but that these will eventually appear (Rothstein, 1975; Dawkins & Krebs, 1979). This hypothesis is often invoked to explain why dunnocks (*Prunella modularis*) fail to recognize odd-looking cuckoo eggs in their nest, since they are parasitized at a low rate (Brooke & Davies, 1988). The evolutionary lag hypothesis also explains why new hosts of cowbirds do not show any of the defences expressed by hosts with a long history of parasitism (such as nest desertion) despite extreme exploitation by their parasite (Hosoi & Rothstein, 2000). However, the evolutionary lag hypothesis is probably impossible to confirm as a cause of a trait's absence because it cannot be easily falsified (Sealy, 1996; Winfree, 1999; Kilner & Langmore, 2011). In fact, it could be considered just an early stage in the arms race leading to the evolution of defences (Stokke *et al.*, 2005).

III. ACCEPTANCE AND VIRULENCE

Each of the processes described above may be influenced by the virulence of the parasite. There is high variation in the damage that a brood parasite can inflict on host fecundity (termed virulence of the parasite *sensu* Kilner, 2005). At one extreme, some cuckoos such as the common cuckoo (*Cuculus canorus*) or Horsfield's bronze-cuckoo (*Chalcites basalis*) are highly virulent and destroy all their host's reproductive success, because the newly hatched cuckoo nestling evicts the hosts' eggs from the nest (Jenner, 1788). Similarly, nestlings of striped cuckoos (*Tapera naevia*) (Morton & Farabaugh, 1979) and honeyguides (Indicatoridae) (Spottiswoode & Koorevaar, 2011) inflict lethal injuries on their companions in the nest, using sharp hooks on the ends of their bills. At the other extreme, black-headed ducks (*Heteronetta atricapilla*) also lay their eggs in nests of other species, but impose little or no costs on their hosts (Lyon & Eadie, 2004). Other brood parasites, including the *Clamator* cuckoos, the parasitic estrildids and the parasitic cowbirds impose intermediate costs on their hosts (Gaston, 1976; Kilner, 2005). Although the hosts invest heavily in rearing these parasites, some can rear their own offspring alongside the parasitic chick. Just as in the case of pathogens, the variation in virulence of brood parasites probably has great implications on the evolutionary outcomes of the host–parasite interaction (Kilner, 2005).

In theory acceptance should never be adaptive in hosts of the most highly virulent parasites. If parasitism results in the loss of all host offspring in a breeding attempt, it should be adaptive to evolve some type of defence no matter how costly it is (unless the defence results in rejection of the host's own young in later breeding attempts, see Lotem, 1993). For example, egg or chick rejection should be selected even if there is a high risk of recognition errors, because accidental rejection of the host's own young is no more costly than acceptance of the parasite (Stokke *et al.*, 2002, 2005). By contrast, in hosts of parasites that are less virulent, rejecting the wrong egg may be more costly, because hosts would lose the chance of raising one of their own eggs, which is still possible if the nest is parasitized (Stokke *et al.*, 2005).

In general, selection for defences should be stronger in hosts of highly virulent parasites. Correspondingly, an analysis based on data provided by Soler (2014) shows that egg rejection is more common in hosts of the more virulent brood parasites than in hosts of cowbirds or *Clamator* cuckoos (less virulent parasites, Fig. 1, $\chi^2 = 8.74$, $P = 0.012$). These data also suggest that the potential for an arms race and evolutionary change in general should be higher in a highly virulent interaction, because defences and counter-defences are likely to evolve more quickly. In fact, a phylogenetically controlled analysis showed that those species of parasites that kill all host progeny are also the species with higher numbers of subspecies, after controlling for geographic range (Krüger, Sorenson & Davies, 2009). This suggests a possible association between virulence and rates of speciation or genetic divergence (Krüger *et al.*, 2009). In tightly coupled host–parasite interactions, such as the case of an arms race (Dawkins & Krebs, 1979), we might expect to find a similar pattern in hosts as well as parasites, namely, more subspecies in hosts of highly virulent parasites.

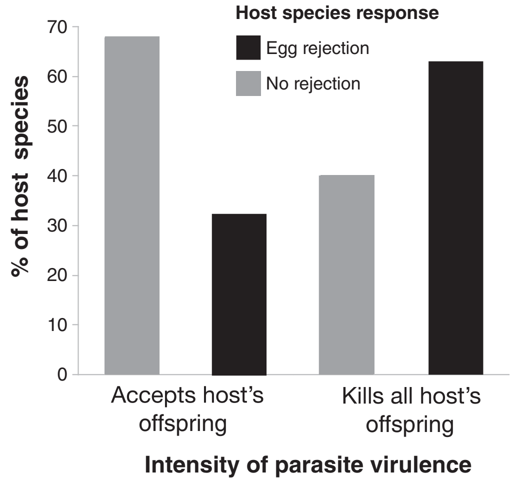


Fig. 1. Egg rejection by hosts is more common (60%, $N = 10$ spp.) in species that are parasitized by highly virulent parasites than in those that are parasitized by non-evicting brood parasites (33%, $N = 31$ spp.). Rejection is assumed when hosts reject >90% of the time, no rejection is assumed when there is <10% of rejection. Data from Soler (2014).

IV. THE EVOLUTION OF TOLERANCE

Tolerance is defined as the ability to minimize the fitness impact of a given parasite burden, not by minimizing the number of enemy attacks (e.g. resistance) but by reducing the impact of the attack (Svensson & Råberg, 2010). It is usually operationally defined by the slope of a regression of host fitness against infection or damage intensity; the steeper the slope, the lower the tolerance (Råberg, Graham & Read, 2009). This means of measuring tolerance is called 'range tolerance' (Little

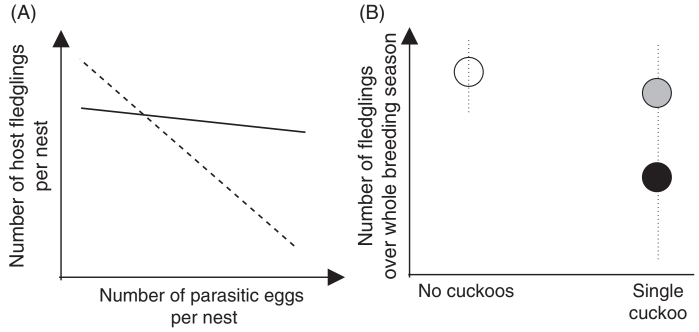


Fig. 2. Schematic view of how range tolerance (A) and point tolerance (B) could be assessed in brood parasitic interactions. In (A) the population represented by the continuous line is more tolerant than that represented by the dashed line. In (B) the population represented by the grey dot is less affected (and more tolerant) than the population represented by the black dot. In this case, both populations are able to lay multiple broods during the breeding season (which is not rare for hosts of brood parasites).

et al., 2010) (Fig. 2A) and has been applied to a study of the interactions between the Eurasian magpie (*Pica pica*) and the great-spotted cuckoo (*Clamator glandarius*) (Soler *et al*., 2011). The relationship between the number of parasitic eggs in a clutch and the number of host fledglings produced varied among magpie populations; populations suffering higher rates of parasitism showed higher tolerance of parasitism (Soler *et al*., 2011). However, in this study individuals that were not parasitized or that rejected an egg were included in the analyses. Since tolerance is measured as the ability to reduce the cost of a given level of parasitism it could be argued that these individuals should have been excluded from the analysis, possibly yielding different outcomes. In this particular example, tolerance is probably favoured over resistance because great-spotted cuckoos destroy the nests of those hosts that show resistance to parasitism (Soler *et al*., 1995). It is important to note that, this study also assumes there is no difference in the overall impact of parasitism among populations.

Another way of measuring tolerance is referred to as ‘point tolerance’ (Fig. 2B); comparison of the fitness of two genotypes at a particular point in time when they both carry the same parasite burden (Little *et al*., 2010). This approach is probably less precise than measuring ‘range tolerance’, because interactions between genotype and parasite burden are obscured (Little *et al*., 2010), but it can be especially useful when it is impossible to construct a continuous axis of parasite burden. In many brood parasites, a single parasite egg is laid in the nest and the parasite chick is reared alone (Davies, 2000), so it is impossible to construct a continuous axis of parasite burden in the nest for a single parasitism event. In this case, comparing fitness at the same level of parasitic burden (e.g. total parasitism) is a good option to test tolerance effectively. However, since all offspring are lost as a result of the parasitism event, fitness would also have to be measured in a different way, such as the total reproductive success at the end of the breeding season for multi-brooded species. This is actually likely to provide a better measure of the fitness costs of parasitism because parasite young are often in the nest for longer than host young, thereby delaying re-nesting in multi-brooded species. Another alternative would be to quantify range tolerance over a different time scale, such as calculating the number of parasitism events over multiple nesting attempts in a breeding season against reproductive success in that season, or the number of parasitism events in a host’s lifetime against lifetime reproductive success. These methods would require that the expression of tolerance by an individual is not correlated with parasitism levels. Quantification of range and point tolerance allows us to distinguish between tolerance and other explanations for acceptance of parasite young. One of the predictions for tolerance is that it covaries geographically with parasite prevalence (Soler *et al*., 2011), such that the costs of rearing parasitic young will be higher for host populations that are rarely or never parasitized than for heavily parasitized populations. This outcome is not predicted for any of the three alternative explanations: strategy blocking, evolutionary equilibrium or evolutionary lag.

The evolution of tolerance may also favour an evolutionary equilibrium between hosts and parasites. An evolutionary equilibrium arises when the costs of defences are greater than the costs of acceptance (see Section II.2). Strategies of tolerance reduce the costs of acceptance, thereby increasing the probability that the costs of defences will outweigh the costs of acceptance and give rise to an evolutionary equilibrium. For example, the splendid fairy-wren (*Malurus splendens*) has evolved a small clutch size and a long breeding season that allows repeated nesting. Under some conditions, these traits, combined with a relatively low cost of parasitism owing to the small size of their primary parasite, the Horsfield’s bronze-cuckoo

(*Chalcites basalis*), reduce the costs of parasitism to the point where the benefits of acceptance are greater than those of rejection (Brooker & Brooker, 1996).

The evolution of tolerance has been widely studied in plants, but less so in animals, and few studies have been conducted on hosts of brood parasites (Svensson & Råberg, 2010). Some studies suggest that hosts may evolve tolerance by adjusting their breeding strategy (Kilner & Langmore, 2011). Where brood parasites are reared alongside host young and host eggs are damaged during parasitism, hosts can minimize the loss of their own young by increasing clutch size. This effect has been demonstrated in both Eurasian magpies (*Pica pica*) (Soler *et al.*, 2001) and Montezuma oropendolas (*Psarocolius montezuma*) (Cunningham & Lewis, 2006). By contrast, when host young rarely survive in parasitized nests, hosts may benefit by adjusting their breeding strategy in the opposite direction and laying smaller clutch sizes (Brooker & Brooker, 1996; Hauber, 2003). A small clutch size, combined with multiple nesting attempts, reduces the risks associated with 'putting all your eggs in one basket', thereby minimizing the costs of parasitism. Hauber (2003) found that hosts of the brown-headed cowbird (*Molothrus ater*) with a long history of parasitism have evolved smaller clutch sizes and more annual breeding attempts than new hosts. Multi-broodedness alone may also be a strategy to reduce the costs of parasitism in grey warblers (*Gerygone igata*), in which populations that suffered higher rates of parasitism had more nesting attempts (Anderson *et al.*, 2013). These studies provide prime opportunities for testing point tolerance; evidence of higher fitness in parasitized individuals with smaller clutch sizes and/or more nesting attempts would suggest that these species have indeed evolved tolerance of brood parasitism.

(1) Simultaneous evolution of tolerance and resistance

Tolerance may evolve as an alternative to resistance, if host defences have been defeated by the parasite or if resistance is too costly to evolve, or it may evolve in addition to lines of resistance (Svensson & Råberg, 2010; Soler *et al.*, 2011). Empirical evidence suggests that plants can allocate resources simultaneously to both strategies (Nunez, Fornoni & Valverde, 2007) and available evidence suggests that the same is true for brood parasite hosts. Soler *et al.* (2001) found evidence that tolerance and resistance were independent adaptive responses to cuckoo parasitism in nine magpie (*Pica pica*) populations. Magpies are parasitized by great-spotted cuckoos (*Clamator glandularis*), which are reared alongside host young. Host eggs are often damaged during parasitism events. Magpies in parasitized populations laid larger clutch sizes (i.e. were more tolerant; Soler *et al.*, 2001) and also rejected non-mimetic eggs more effectively, suggesting that there was no trade-off between the two strategies. Similarly, in a comparison of nine magpie populations, those with higher levels of resistance also showed higher tolerance of parasitism (Soler *et al.*, 2011). This evidence suggests that resistance and tolerance are not exclusive strategies and that they can be present within the same population and the same individuals. In plant–herbivore interactions, it is not uncommon for plant species to express resistance traits such as antibiosis combined with tolerance strategies such as increased seed production (Fornoni, Valverde & Nunez, 2003; Leimu & Koricheva, 2006). Furthermore, there is some evidence in birds that tolerance has coevolved with resistance; in Montezuma oropendolas (*Psarocolius montezuma*), for example, females maximize their reproductive success through a combination of resistance, in the form of rejection of parasite eggs, and tolerance, by laying an 'insurance egg' to compensate for egg loss caused by the parasite (Cunningham & Lewis, 2006). If the nest is not parasitized and the insurance egg hatches, the chick is rejected by its parents. Only through expression of both resistance and tolerance can a parasitized nest succeed in producing host young.

(2) How does selection act on tolerance?

Selection acting on tolerance and resistance can also differ. In plants, traits related to tolerance and resistance are selected by factors other than herbivory or fungal pathogens (Nunez *et al.*, 2007). For instance, many tolerance- but not resistance-related traits in plants are associated with growth rate, which is well known to respond to other selective pressures such as desiccation or trampling (Fornoni *et al.*, 2003; Nunez *et al.*, 2007). The same idea could be extrapolated to brood parasitism. In general, strategies of resistance are quite specific to interactions with brood parasites (e.g. mobbing of adult brood parasites, rejection of parasite eggs or chicks), whereas strategies of tolerance often entail modification of breeding strategies (e.g. clutch size, frequency of nesting; Kilner & Langmore, 2011), traits that are typically under strong natural selection. For instance, nest predation and seasonality are significantly related to clutch size (Jetz, Sekercioglu & Bohning-Gaese, 2008; Griebeler, Caprano & Bohning-Gaese, 2010). The fact that tolerance traits are probably under more diverse selective pressures might have implications for their evolution and stabilization. In this sense brood parasitic systems offer an advantage as a model system for studies of tolerance compared to endoparasitic systems, because there is less potential for confounding effects when testing a trait as a tolerance mechanism, given that in endoparasitic systems the parasite has a direct impact on the physiology of the host making it difficult to isolate traits.

(3) Evolutionary outcomes of tolerance

Whether hosts tolerate or resist parasitism will have important evolutionary implications for the ecological feedbacks of the interaction (Miller, White & Boots, 2006; Boots, 2008). Tolerance, by definition, does not impose any direct costs on the enemy; accordingly, it will not induce selection for counter-adaptations and will not lead to antagonistic coevolution (Svensson & Råberg, 2010). By contrast, resistance traits directly threaten parasite fitness, and thereby select for parasite counter-adaptations, leading to escalating or cyclical coevolutionary arms races (Spottiswoode & Stevens, 2011). In plants, tolerance tends to reduce genetic variation within populations (Fornoni, 2011) and tolerance strategies are easily fixed (Roy & Kirchner, 2000). Thus, communities of plants with a long history of herbivores are dominated by tolerant species and are less diverse (Fornoni, 2011). If we extrapolate this theory to the context of brood parasitism we would predict that, whenever tolerance is adaptive, strategies of tolerance (e.g. small clutch size) should be more widespread and prevalent across hosts than equally successful strategies of resistance (e.g. egg rejection). This would arise because brood parasites would not evolve counter-adaptations in response to a reduced clutch size, since there is no decrease in fitness of the parasite. By contrast, brood parasites evolve counter-adaptations to the evolution of egg rejection in hosts (such as egg mimicry or destruction of host nests), which hinder the spread and establishment of unique resistance strategies in hosts.

Moreover, studies on host–pathogen interactions have shown that tolerance may compensate completely for the damage caused by the parasites, leading to the evolution of commensalism (Little *et al.*, 2010). One possible example of this is provided by the brood parasitic black-headed duck (*Heteronetta atricapilla*) (Rees & Hillgarth, 1984). The precocious ducklings of this species do not require post-hatching parental care from their main host, red-gartered coots (*Fulica armillata*), and parasitism does not reduce clutch size or hatching success of host eggs in this particular species, nor does it increase nest predation rates (Lyon & Eadie, 2004). However, it is not currently known whether adaptations for tolerance in the host contributed to the absence of parasitism costs in this system. Additionally, commensalism can be achieved not only through hosts that tolerate their parasites, but also through parasites that evolve to reduce their virulence to a level that is not damaging to the host (Miller, White & Boots, 2005; Miller *et al.*, 2006; Oliver, Leather & Cook, 2009). There are still few examples of tolerance in the brood parasitism literature and, although it has been demonstrated that parasitism can be less costly for some populations than for others, the mechanisms behind tolerance are very poorly explored (Soler *et al.*, 2011).

(4) Where to look for tolerance?

In host–pathogen systems an increase in host tolerance may result in a corresponding increase in the prevalence of parasites in the population (Miller *et al.*, 2006). In the case of brood parasitism, more parasites will be raised successfully in nests of tolerant populations than in resistant populations, increasing the number of parasitic individuals and possibly increasing the frequency of parasitism. Studying populations with high parasitism prevalence and apparent lack of defences might be the key to finding more examples of tolerance in brood parasitism systems. For instance, populations of magpies (*Pica pica*) in Europe showed a positive association between levels of tolerance to brood parasitism and parasite prevalence (Soler *et al.*, 2011). Hence, populations with high levels of parasitism might not only be the product of parasites defeating the host (Hoover & Robinson, 2007), but could also indicate that the population has evolved tolerance.

V. CONCLUSIONS

(1) A lack of defences in brood parasite hosts is usually considered an evolutionary anomaly that can be explained by three non-mutually exclusive hypotheses: evolutionary lag, evolutionary equilibrium and strategy blocking.

(2) Hosts will be more or less likely to evolve defences depending on the virulence of their parasite. Egg rejection is more prevalent in hosts of highly virulent parasites than in hosts of non-evicting parasites.

(3) Resistance is not the only adaptive response to parasitism; hosts can also evolve tolerance, where they reduce the costs of being parasitized by adjusting life-history traits.

(4) Strategies of tolerance and resistance may occur as a mixed strategy and may coevolve to maximize fitness. It is important to study both when assessing the defence portfolio of a population.

(5) Types of defences (resistance *versus* tolerance) have different evolutionary outcomes that have the potential to generate phylogenetically traceable footprints (e.g. affect diversity); an interesting avenue for future study.

(6) The interactions between plants and herbivores, hosts and pathogens, and between hosts and brood parasites show marked parallels, providing opportunities to apply the theoretical framework developed for the study of these to brood parasite–host interactions. Moreover, the interactions between brood parasites and their hosts offer much potential for studies of the evolution of tolerance.

VI. ACKNOWLEDGEMENTS

We are grateful to Carlos Bustos, Celeste Linde and two anonymous reviewers for very helpful comments and suggestions on the manuscript. N.E.L. was supported by the Australian Research Council.

VII. REFERENCES

Anderson, M. G., Gill, B. J., Briskie, J. V., Brunton, D. H. & Hauber, M. E. (2013). Latitudinal differences in the breeding phenology of grey warblers covary with the relationship to the prevalence of parasitism by shining bronze cuckoos. *Emu* **113**, 187–191.

Anderson, M. G., Ross, H. A., Brunton, D. H. & Hauber, M. E. (2009). Begging call matching between a specialist brood parasite and its host: a comparative approach to detect coevolution. *Biological Journal of the Linnean Society* **98**, 208–216.

Antonov, A., Stokke, B. G., Moksnes, A. & Røskaft, E. (2009). Evidence for egg discrimination preceding failed rejection attempts in a small cuckoo host. *Biology Letters* **5**, 169–171.

Aviles, 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. *Journal of Evolutionary Biology* **19**, 1901–1910.

Boots, M. (2008). Fight or learn to live with the consequences? *Trends in Ecology and Evolution* **23**, 248–250.

Briskie, J. V., Sealy, S. G. & Hobson, K. A. (1992). Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution* **46**, 334–340.

Britton, N., Planqué, R. & Franks, N. (2007). Evolution of defence portfolios in exploiter–victim systems. *Bulletin of Mathematical Biology* **69**, 957–988.

Brooke, M.d. L. & Davies, N. B. (1988). Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* **335**, 630–632.

Brooker, M. G. & Brooker, L. C. (1989). The comparative breeding behaviour of two sympatric cuckoos, Horsfield's Bronze-Cuckoo *Chrysococcyx basalis* and the Shining Bronze-Cuckoo *C. lucidus*, in Western Australia: a new model for the evolution of egg morphology and host specificity in avian brood parasites. *Ibis* **131**, 528–547.

Brooker, M. G. & Brooker, L. C. (1991). Eggshell strength in cuckoos and cowbirds. *Ibis* **133**, 406–413.

Brooker, M. & Brooker, L. (1996). Acceptance by the splendid fairy-wren of parasitism by Horsfield's bronze-cuckoo: further evidence for evolutionary equilibrium in brood parasitism. *Behavioral Ecology* **7**, 395–407.

Canestrari, D., Bolopo, D., Turlings, C. J., Roder, G., Marcos, J. M. & Baglione, V. (2014). From parasitism to mutualism: unexpected interactions between a cuckoo and its host. *Science* **343**, 1350–1352.

Canestrari, D., Marcos, J. M. & Baglione, V. (2009). Cooperative breeding in carrion crows reduces the rate of brood parasitism by great spotted cuckoos. *Animal Behaviour* **77**, 1337–1344.

Carro, M. E. & Fernandez, G. J. (2013). Can nest predation explain the lack of defenses against cowbird brood parasitism in the rufous-collared sparrow (*Zonotrichia capensis*)? *The Auk* **130**, 408–416.

Cunningham, E. J. & Lewis, S. (2006). Parasitism of maternal invesment selects for increased clutch size and brood reduction in a host. *Behavioural Ecology* **17**, 126–131.

Davies, N. B. (1989). Cuckoos and parasitic ants: brood parasitism as an evolutionary arms race. *Journal of Zoology* **217**, 521–522.

Davies, N. B. (1999). Cuckoos and cowbirds versus hosts: co-evolutionary lag and equilibrium. *Ostrich: Journal of African Ornithology* **70**, 71–79.

Davies, N. B. (2000). *Cuckoos, Cowbirds and other Cheats*. T. & A.D. Poyser, London.

Davies, N. B. & Brooke, M.d. L. (1988). Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour* **36**, 262–284.

Davies, N. B., Brooke, M. D. L. & Kacelnik, A. (1996). Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **263**, 925–931.

Dawkins, R. & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **205**, 489–511.

De Mársico, M. C., Gantchoff, M. G. & Reboreda, J. C. (2012). Host–parasite coevolution beyond the nestling stage? Mimicry of host fledglings by the specialist screaming cowbird. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **279**, 3401–3408.

De Marsico, M. C., Gloag, R., Ursino, C. A. & Reboreda, J. (2013). A novel method of rejection of brood parasitic eggs reduces parasitism intensity in a cowbird host. *Biology Letters* **9**, 200130076.

Edvardsen, E., Moksnes, A., Røskaft, E., Øien, I. J. & Honza, M. (2001). Egg mimicry in cuckoos parasitizing four sympatric species of *Acrocephalus* warblers. *The Condor* **103**, 829–837.

Feeney, W. E. & Langmore, N. E. (2013). Social learning of a brood parasite by its host. *Biology Letters* **9**, 20130443.

Feeney, W. E., Medina, I., Somveille, M., Heinsohn, R., Hall, M. L., Mulder, R. A., Stein, J. A., Kilner, R. M. & Langmore, N. E. (2013). Brood parasitism and the evolution of cooperative breeding in birds. *Science* **342**, 1506–1508.

Feeney, W. E., Welbergen, J. A. & Langmore, N. E. (2012). The front-line of avian brood parasite-host coevolution. *Animal Behaviour* **84**, 3–12.

Foitzik, S., Fischer, B. & Heinze, J. (2003). Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behavioral Ecology* **14**, 80–88.

Fornoni, J. (2011). Ecological and evolutionary implications of plant tolerance to herbivory. *Functional Ecology* **25**, 399–407.

Fornoni, J., Valverde, P. L. & Nunez, J. (2003). Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of Datura stramonium. *Evoluionary Ecology Research* **5**, 1049–1065.

Gaston, A. J. (1976). Brood parasitism by the pied crested cuckoo *Clamator jacobinus*. *Journal of Animal Ecology* **45**, 331–348.

Gloag, R., Fiorini, V. D., Reboreda, J. C. & Kacelnik, A. (2011). Brood parasite eggs enhance egg survivorship in a multiply parasitized host. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **279**, 1831–1840.

Gloag, R., Keller, L. A. & Langmore, N. E. (2014). Cryptic cuckoo eggs hide from competing cuckoos. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **281**, 1–7.

Griebeler, E. M., Caprano, T. & Bohning-Gaese, K. (2010). Evolution of avian clutch size along latitudinal gradients: do seasonality, nest predation or breeding season length matter? *Journal of Evolutionary Biology* **23**, 888–901.

Grim, T. (2007). Experimental evidence for chick discrimination without recognition in a brood parasite host. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **274**, 373–381.

Grim, T., Samaš, P., Moskát, C., Kleven, O., Honza, M., Moksnes, A., Røskaft, E. & Stokke, B. G. (2011). Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? *Journal of Animal Ecology* **80**, 508–518.

Hauber, M. E. (2003). Interspecific brood parasitism and the evolution of clutch sizes. *Evolutionary Ecology Research* **5**, 559–570.

Hobson, K. A. & Sealy, S. G. (1989). Responses of yellow warblers to the threat of cowbird parasitism. *Animal Behaviour* **38**, 510–519.

Honza, M., Prochazka, P., Morongova, K., Capek, M. & Jelinek, V. (2011). Do nest light conditions affect rejection of parasitic eggs? A test of the light environment hypothesis. *Ethology* **117**, 538–546.

Hoover, J. P. & Robinson, S. K. (2007). Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4479–4483.

Hosoi, S. A. & Rothstein, S. I. (2000). Nest desertion and cowbird parasitism: evidence for evolved responses and evolutionary lag. *Animal Behaviour* **59**, 823–840.

Jenner, E. (1788). Observations on the natural history of the cuckoo. By Mr. Edward Jenner. In a letter to John Hunter, Esq. F.R.S. *Philosophical Transactions of the Royal Society of London* **78**, 219–237.

Jetz, W., Sekercioglu, C. H. & Bohning-Gaese, K. (2008). The worldwide variation in avian clutch size across species and space. *PLoS Biology* **6**, e303.

Kilner, R. M. (2005). The evolution of virulence in brood parasites. *Ornithological Science* **4**, 55–64.

Kilner, R. M. (2006). The evolution of egg colour and patterning in birds. *Biological Reviews* **81**, 383–406.

Kilner, R. M. & Langmore, N. E. (2011). Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biological Reviews of the Cambridge Philosophical Society* **86**, 836–852.

Krüger, O. (2011). Brood parasitism selects for no defence in a cuckoo host. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **278**, 2777–2783.

Krüger, O., Sorenson, M. D. & Davies, N. B. (2009). Does coevolution promote species richness in parasitic cuckoos? *Proceedings of the Royal Society of London, Series B: Biological Sciences* **276**, 3871–3879.

Langmore, N. E., Cockburn, A., Russell, A. F. & Kilner, R. M. (2009a). Flexible cuckoo chick-rejection rules in the superb fairy-wren. *Behavioural Ecology* **20**, 978–984.

Langmore, N. E., Stevens, M., Maurer, G. & Kilner, R. M. (2009b). Are dark cuckoo eggs cryptic in host nests? *Animal Behaviour* **78**, 461–468.

Langmore, N. E. & Kilner, R. M. (2010). The coevolutionary arms race between Horsfield's Bronze-Cuckoos and Superb Fairy-wrens. *Emu* **110**, 32–38.

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. *Behavioral Ecology* **16**, 686–692.

Langmore, N. E., Maurer, G., Adcock, G. J. & Kilner, R. M. (2008). Socially acquired host-specific mimicry and the evolution of host races in Horsfield's bronze-cuckoo *Chalcites basalis*. *Evolution* **62**, 1689–1699.

Langmore, N. E., Stevens, M., Maurer, G., Heinsohn, R., Hall, M. L., Peters, A. & Kilner, R. M. (2011). Visual mimicry of host nestlings by cuckoos. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **278**, 2455–2463.

Leimu, R. & Koricheva, J. (2006). A meta-analysis of traddeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos* **112**, 1–9.

Little, T. J., Shuker, D. M., Colegrave, N., Day, T. & Graham, A. L. (2010). The coevolution of virulence: tolerance in perspective. *PLoS Pathogens* **6**, e1001006.

Lotem, A. (1993). Learning to recognize nestlings is maladaptive for cuckoo *Cuculus canorus* hosts. *Nature* **362**, 743–745.

Lotem, A., Nakamura, H. & Zahavi, A. (1992). Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behavioral Ecology* **3**, 128–132.

Lotem, A., Nakamura, H. & Zahavi, A. (1995). Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour* **49**, 1185–1209.

Lyon, B. E. & Eadie, J. M. (2004). An obligate brood parasite trapped in the intraspecific arms race of its hosts. *Nature* **432**, 390–393.

Marchetti, K. (2000). Egg rejection in a passerine bird: size does matter. *Animal Behaviour* **59**, 877–883.

Miller, M. R., White, A. & Boots, M. (2005). The evolution of host resistance: tolerance and control as distinct strategies. *Journal of Theoretical Biology* **236**, 198–207.

Miller, M. R., White, A. & Boots, M. (2006). The evolution of parasites in response to tolerance in their hosts: the good, the bad and apparent commensalism. *Evolution* **60**, 945–956.

Moksnes, A., Røskaft, E. & Korsnes, L. (1993). Rejection of cuckoo (*Cuculus canorus*) eggs by meadow pipits (*Anthus pratensis*). *Behavioral Ecology* **4**, 120–127.

Morton, E. S. & Farabaugh, S. M. (1979). Infanticide and other adaptations of the nestling striped cuckoo *Tapera naevia*. *Ibis* **121**, 212–213.

Moskát, C., Szentpéteri, J. & Barta, Z. (2002). Adaptations by great reed warblers to brood parasitism: a comparison of populations in sympatry and allopatry with the common cuckoo. *Behaviour* **139**, 1313–1329.

Nunez, J., Fornoni, J. & Valverde, P. L. (2007). The evolution of resistance and tolerance to herbivores. *Annual Reviews in Ecology and Evolution* **38**, 541–566.

Oliver, T. H., Leather, S. R. & Cook, J. M. (2009). Tolerance traits and the stability of mutualism. *Oikos* **118**, 346–352.

Petit, L. J. (1991). Adaptive tolerance of cowbird parasitism by prothonotary warblers: a consequence of nest-site limitation? *Animal Behaviour* **41**, 425–432.

Picman, J. & Pribil, S. (1997). Is greater eggshell density an alternative mechanism by which parasitic cuckoos increase the strength of their eggs? *Journal of Ornithology* **138**, 531–541.

Planqué, R., Britton, N. F., Franks, N. R. & Peletier, M. A. (2002). The adaptiveness of defence strategies against cuckoo parasitism. *Bulletin of Mathematical Biology* **64**, 1045–1068.

Råberg, L., Graham, A. L. & Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **364**, 37–49.

Redondo, T. & Reyna, L. A. D. (1988). Vocal mimicry of hosts by great spotted cuckoo *Clamator glandarius*: further evidence. *Ibis* **130**, 540–544.

Rees, E. & Hillgarth, N. (1984). The breeding biology of captive black-headed ducks and the behaviour of their young. *Condor* **86**, 242–250.

Rothstein, S. I. (1975). Experimental and teleonomic investigation of avian brood parasitism. *Condor* **77**, 250–271.

Roy, B. A. & Kirchner, J. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**, 51–63.

Sato, N. J., Tokue, K., Noske, R. A., Mikami, O. K. & Ueda, K. (2010). Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. *Biology Letters* **6**, 67–69.

Sealy, S. G. (1996). Evolution of host defenses against brood parasitism: implications of puncture-ejection by a small passerine. *The Auk* **113**, 346–355.

Smith, N. G. (1968). The advantage of being parasitized. *Nature* **219**, 690–694.

Smith, J. N. M., Arcese, P. & McLean, I. G. (1984). Age, experience, and enemy recognition by wild song sparrows. *Behavioral Ecology and Sociobiology* **14**, 101–106.

Soler, M. (2014). Long-term coevolution between avian brood parasites and their hosts. *Biological Reviews* **89**, 688–704.

Soler, J. J., Martinez, J. G., Soler, M. & Moller, A. (2001). Life history of magpie populations sympatric or allopatric with the brood parasitic great spotted cuckoo. *Ecology* **82**, 1621–1631.

Soler, J. J., Martín-Gálvez, D., Martínez, J. G., Soler, M., Canestrari, D., Abad-Gómez, J. M. & Møller, A. P. (2011). Evolution of tolerance by magpies to brood parasitism by great spotted cuckoos. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **278**, 2047–2052.

Soler, J. J. & Soler, M. (2000). Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia* **125**, 309–320.

Soler, M., Soler, J. J., Martinez, J. G. & Moller, A. P. (1995). Magpie host manipulation by great spotted cuckoos: evidence for an avian mafia? *Evolution* **49**, 770–775.

Spottiswoode, C. N. & Koorevaar, J. (2011). A stab in the dark: chick killing by brood parasitic honeyguides. *Biology Letters* **8**, 241–244.

Spottiswoode, C. N. & Stevens, M. (2011). How to evade a coevolving brood parasite: egg discrimination versus egg variability as host defences. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **1724**, 3566–3573.

Stoddard, M. C. & Stevens, M. (2010). Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **277**, 1387–1393.

Stoddard, M. C. & Stevens, M. (2011). Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution* **65**, 2004–2013.

Stokke, B. G., Honza, M., Moksnes, A., Skaft, E. & Rudolfsen, G. (2002). Costs associated with recognition and rejection of parasitic eggs in two European passerines. *Behaviour* **139**, 629–644.

Stokke, B. G., Moksnes, A. & Røskaft, E. (2005). The enigma of imperfect adaptations in hosts of avian brood parasites. *Ornithological Science* **4**, 17–29.

Svensson, E. I. & Råberg, L. (2010). Resistance and tolerance in animal enemy-victim coevolution. *Trends in Ecology & Evolution* **25**, 267–274.

Thorogood, R. & Davies, N. B. (2012). Cuckoos combat socially transmitted defenses of reed warbler hosts with a plumage polymorphism. *Science* **337**, 10–25.

Tokue, K. & Ueda, K. (2010). Mangrove gerygones *Gerygone laevigaster* eject little bronze-cuckoo *Chalcites minutillus* hatchlings from parasitized nests. *Ibis* **152**, 835–839.

Welbergen, J. A. & Davies, N. B. (2011). A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behavioral Ecology* **22**, 574–579.

Winfree, R. (1999). Cuckoos, cowbirds and the persistence of brood parasitism. *Trends in Ecology and Evolution* **14**, 338–343.

Zahavi, A. (1979). Parasitism and nest predation in parasitic cuckoos. *The American Naturalist* **113**, 157–159.

(*Received 28 July 2014; revised 26 January 2015; accepted 12 February 2015*)

Chapter 2

The evolution of clutch size in hosts of avian brood parasites

This chapter was done in collaboration with other researchers. My supervisor and I conceived the project idea, and I performed the comparative analysis and wrote the paper. The paper has been resubmitted with revisions to the journal *Evolution*.

The evolution of clutch size in hosts of avian brood parasites

Iliana Medina[1*], Naomi E. Langmore[1], Robert Lanfear[1,2], and Hanna Kokko[1,3].

[1]Department of Ecology, Evolution and Genetics, Research School of Biology, Australian National University, Canberra.

[2]Department of Biological Sciences, Macquarie University, Sydney, Australia.

[3]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, Zurich 8057, Switzerland.

Abstract

Coevolution with avian brood parasites shapes a range of traits in their hosts, including morphology, behaviour and breeding systems. Here we explore whether brood parasitism is also associated with the evolution of host clutch size. Several studies have proposed that hosts of highly virulent parasites could decrease the costs of parasitism by evolving a smaller clutch size, because hosts with smaller clutches will lose fewer progeny when their clutch is parasitised. We describe a model of the evolution of clutch size in hosts of brood parasites, which challenges this logic, and shows instead that an increase in clutch size (or no change) should evolve in hosts. We test this prediction using a broad-scale comparative analysis to ask whether there are differences in clutch size between hosts and non-hosts. Our results support the model and show that this effect is more pronounced with increasing parasite virulence; host species that incur in larger costs from raising a parasite, lay larger clutches. We conclude that brood parasitism may select for the evolution of larger clutches in hosts, and that brood parasitism can be an influential factor in clutch size evolution.

Introduction

Interspecific brood parasites such as cuckoos (Cuculidae), cowbirds (Icteridae) and honeyguides (Indicatoridae) lay their eggs in the nests of other species, avoiding the costs of raising their own offspring (Davies 2000). The high costs of parasitism have led to the evolution of defence strategies in hosts, which in turn select for reciprocal strategies in parasites to evade host defences (Davies 1999; Davies 2000, 2011). Brood parasitism affects hundreds of host species worldwide, and has shaped the evolution of numerous host traits, including egg phenotype (Stokke et al. 2002; Yang et al. 2010; Stoddard and Stevens 2011), nestling morphology (Payne 2005; Hauber and Kilner 2007) and breeding systems (Feeney et al. 2013).

Avian clutch size (the number of eggs laid per nest) is a central life-history variable, being a major determinant of avian reproductive effort and one of the best-recorded animal life-history traits (Jetz et al. 2008). For years ecologists have tried to explain the huge variation in clutch size among birds, and it is now well known that variables such as latitude, nest type and mortality risk are important predictors (Jetz et al. 2008; Samas et al. 2013; Martin 2015). In this study we test one potential way in which brood parasitism could be associated with the clutch size of bird species.

The tolerance hypothesis

Studies of avian brood parasitism have focused largely on strategies of resistance in hosts, such as rejection of brood parasite eggs and chicks and mobbing of adult brood parasites (Langmore et al. 2003; Davies and Welbergen 2009; Sato et al. 2010; Spottiswoode and Stevens 2011). In all these cases hosts decrease the success of the parasite. However, there is some evidence that hosts can also evolve tolerance towards brood parasites (Soler et al. 2011). Tolerance is a broad term that refers to the ability of a victim of a negative interspecific interaction to compensate for the damage. For example, plants can evolve tolerance to being partially eaten by herbivores through compensatory growth (Svensson and Råberg 2010). Likewise disease tolerance refers to the ability of organisms to maintain fitness components at an adequate level if resistance to acquiring the disease in the first place has failed (Cronin et al. 2014). In the context of avian brood parasitism, tolerance means that hosts adjust their life history and morphological traits to minimize the impact of parasitism on their fitness (Brooker and Brooker 1996; Svensson and Råberg 2010; Kilner and Langmore 2011; Medina and Langmore 2015b). For example, Soler et al. (2011) showed that some populations of magpies can experience an increase in the number of parasitic eggs in their nests without detrimental fitness effects, indicating that tolerance has evolved in this species. Tolerance differs from strategies of resistance in that it does not impose clear costs on the parasite: parasite young are raised to independence.

Little is known about the evolution of tolerance in hosts of brood parasites (Medina and Langmore 2015b). Adjustment of clutch size has been proposed as a mechanism to minimize the impact of parasitism, but clutch size may be predicted to increase or decrease, depending on whether the parasite chick kills the host young or is reared alongside them. In some brood parasites, such as the parasitic cowbirds (*Molothrus spp.*) or *Clamator* spp. cuckoos, the parasitic chick does not evict host young from the nest ('non-evictors', Kilner 2005). In this case, it has been suggested that hosts should evolve larger clutch sizes to 'dilute' the costs of damage to host eggs caused by the parasite (Cunningham and Lewis 2006). This means that a host will have a larger number of undamaged eggs after a single parasitism event, as has been demonstrated in Eurasian magpies *Pica pica* (Soler et al. 2001) and Montezuma oropendolas, *Psacorolius montezuma*, (Cunningham and Lewis 2006). However, if hosts can raise all these young to independence (a larger number of their own, and the parasite) such that it pays off despite potential costs to the parent, it is not clear why selection would not favour a corresponding or even larger increase in the *absence* of parasitism: after all, the quantity maximized by selection is lifetime reproductive success, and not proxies such as the ratio of host to parasite young raised by the parent. The answer may be that hosts *cannot* raise all the young to independence, and instead practice brood reduction in unparasitized nests (Lerkelund et al. 1993). This is the case in the Montezuma oropendola (*Psarocolius montezuma*), which lay a second egg purely as insurance against brood parasitism; if the nest is not parasitized the second chick never survives beyond three days (Cunningham and Lewis, 2006). However, if the nest is parasitized, resulting in loss of one host egg, the host typically rejects the parasite egg, leaving it with one viable egg of its own in the nest. Thus it seems that an enlarged clutch size is adaptive in some hosts of non-evicting brood parasites.

By contrast, in hosts of evicting parasites, it has been argued that brood parasitism should select for reduced clutch size when brood parasitism results in the loss of the host brood, although this hypothesis is more contentious. Parasitism by the majority of cuckoos, honeyguides and some cowbirds results in the death of the host young, either through eviction of host eggs, attack on host nestlings by the parasite chick, or through starvation of host nestlings (Kilner 2005). Here, life history theory has been used to argue that the increased risk of juvenile mortality should select for decreased investment in each reproductive attempt (e.g. a smaller number of eggs (Hauber 2003; Servedio and Hauber 2006). The idea that brood parasitism selects for a smaller clutch size has been suggested explicitly for superb fairy-wrens *Malurus cyaneus* (Brooker and Brooker 1996) and hosts of cowbirds (Hauber 2003). This argument is intuitively plausible: laying many small clutches rather than few, large clutches means that if a clutch is parasitized fewer eggs will be lost. This could conceivably lead to a higher lifetime reproductive success.

Despite its intuitive appeal, the argument that a bird can reduce the costs of parasitism by laying many, small clutches rather than few, large clutches involves flawed logic, which can be illustrated with a hypothetical example (Figure 1). Consider that the tolerance strategy involves reducing the clutch size from 5 to 4, which saves reproductive effort so that the parent now achieves on average one extra brood: in the example it shifts from four to five broods in a lifetime on average (Figure 1A). At first sight this example seems to support the logic of tolerance. The presence of parasites shifts the tolerance-related (slow) life history from being no better than the faster strategy — both achieving 20 offspring— to apparently advantageous, with the tolerant (slow) strategy achieving 16 young, and the fast strategy 15 (Figure 1B) where parasites destroy some broods.

However, this comparison assumes that there is only one parasitism attempt per lifetime. In reality, producing a larger number of broods (the slow life history with its many small clutches) will also present brood parasites with more opportunities to parasitise. If we assume that tolerant phenotypes are not also more resistant, we cannot assume that the additional brood on the panel B of figure 1 will remain unparasitized. Correcting for this mistake requires taking into account that any brood may be parasitised, including any new ones that arise as a result of a slowing down the tolerant phenotype's reproductive rate.

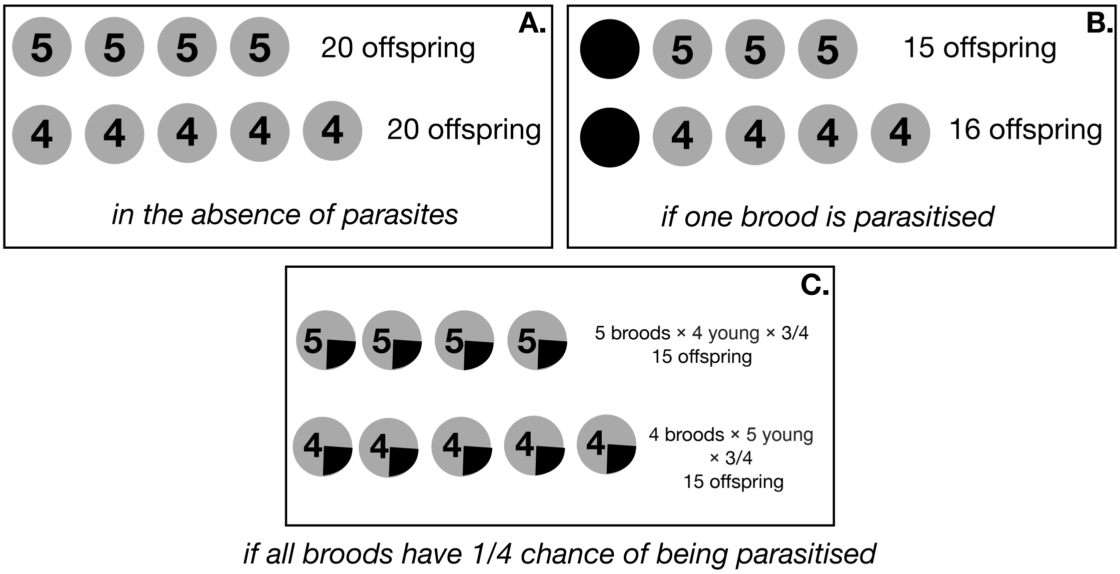


Figure 1. Representation of decreased clutch size as a tolerance mechanism and why the underlying rationale is flawed (see text for explanation). On panel A there is no parasitism, on panel B one brood is parasitised by a highly virulent parasite (e.g. a cuckoo) and on panel C each brood has the same probability (1/4) of being parasitised.

Thus, if we correct the assumption such that the level of parasitism for the non-tolerant phenotype (1 out of 4 on average lost) also applies to the tolerant one (Figure 1C), then the reproductive success of the tolerant type has to be revised to ¼ of all the 5 broods lost, which means a total of 1.25 broods lost and 3.75 completed in a lifetime. The expected total number of offspring it raises to independence is now 3.75 × 4 = 15, in other words no improvement at all over the non-tolerant strategy: the tolerant phenotype is still neutral now that parasitism probability has been added.

It appears that these calculations, and its generalizations, have not been performed in the literature thus far, despite some literature superficially giving this appearance (Hauber 2003; Servedio and Hauber 2006). For example, Servedio & Hauber (2006) model the evolution of egg rejection (not tolerance), but cite others for tolerance — yet none of the five articles cited actually model the question: Partridge & Harvey (1985) provide a verbal description of cost of reproduction in general, Oppliger et al. (1996) present an experiment testing whether egg production is costly, and Lyon (1998) considers evolution of the parasite's (not host's) clutch size in species with conspecific brood parasitism — a clearly different research question from the evolution of tolerance as a response to interspecific brood parasitism. Finally, Martin et al. (2001) present a relatively complex argument based on immunocompetence, and its predictions are produced verbally. Hauber (2003) additionally cites Forbes & Lamey (1996) for a quantitative model, but again, the purpose of the original model differs from its later interpretive use: Forbes & Lamey were interested in the question of parents producing an 'insurance egg' that usually will not yield fitness but can nevertheless elevate the expected brood success to above zero should the primary brood member die e.g. because of developmental failures. One can consider the fit between these assumptions and most cases of interspecific parasitism to be relatively poor.

Thus, it appears that while other types of predictions are checked with theoretical development (e.g. evolution of clutch size of parasites, Lyon 1998), we do not yet have a key model relating to the verbal predictions according to which hosts of highly virulent parasites are predicted to evolve a reduced clutch size. Our first aim is to provide this missing theory, avoiding the pitfall of Figure 1, by modelling the evolution of clutch size in hosts of evicting brood parasites, such as cuckoos.

Our second aim is to test whether hosts do indeed have smaller clutches than non-host species, by performing a comparative analysis of clutch size across 829 species of hosts and non-hosts from America, Australia, Africa and Europe.

Methods

The model

The aim of our model is to compare two options for parasitized species (hosts): they may either tolerate the parasitism and accept all eggs regardless of origin (the *accepting strategy*, denoted A), or exhibit rejection behaviours (the *rejecting strategy*, denoted R). We first derive the best clutch size assuming that the prevailing strategy is either A or R, and denote these F_A* and F_R*, respectively; then we ask if A strategists using F_A* have higher fitness than R strategists using F_R*. We then assume that the inferior strategy (either A or R) does not evolve, and proceed to evaluating the key question: how does the predicted clutch size of the winning strategy compare with those of unparasitized species with otherwise identical life history constraints? This last comparison assumes that the unparasitized species, i.e. a non-host, does not perform rejecting behaviours, and we thus use the same optimization procedure to derive its optimal clutch size as we use for A strategists, but with the rate of parasitism set to zero.

The answers to the above questions depend on the rate of parasitism (denoted P), the probabilities of false positives and negatives (α and β, respectively) when detecting foreign eggs, and the costs of raising con- and heterospecific young (parameters a, b, C, k and K specify the life history in the presence of these costs; for details see Supplementary Material). The evolutionary outcomes also depend on how we assume hosts to perform rejection behaviours. We thus perform the above comparisons for two different scenarios. In the first scenario, called egg rejection, hosts that reject an egg eject this egg only from a nest. False positives mean that an own egg will be ejected. The probability of a false positive (ejecting an egg that was one's own) is the same and independent for each own egg. In scenario 2, nest abandonment, rejecting means abandoning an entire clutch, and this also leads to the death of the host's own offspring from the current breeding attempt.

Phylogenetic Comparative Analysis

Species used

We chose 104 Australian passerine species based on the Brooker and Brooker's (1989) classification of (i) species that are unsuitable as hosts and (ii) biological hosts of eight species of Australian cuckoos (see Supplementary Material). We excluded introduced species, and species recorded as occasional hosts, because we cannot infer the extent of coevolution with cuckoos in these species. For American species (n=89) we used the published data by Soler (2014) and Friedmann and Kiff (1985) of hosts of cowbirds and we excluded the 'rare host' category, or those species that had less than 10% of their nests parasitised, because it was not possible to infer the extent of selection on these hosts by brood parasites. For analyses of southern African species (n=636), we used the host listing for all brood parasites in 'Roberts Birds of Southern Africa' (Hockey et al. 2005). We included all species listed as hosts of honeyguides, cuckoos, and Vidua finches, as well as non-host species, and excluded those listed as provisional hosts. This list includes non-passerine hosts, which are parasitized by honeyguides (Indicatoridae), and some hosts that migrate to Europe or are also present in Europe (taken from Soler, 2014).

Data collection

Information on the average clutch size for each species was obtained from the supplementary material from Jetz et al. (2008). Clutch size varies with latitude, nest type and body size (Jetz et al. 2008). To control for potentially confounding effects of latitude, we used the R package *dismo* to download 2000 random records per species from the GBIF (Hijmans et al. 2011), and calculated the median latitude of the records. This value represents where the species is more often recorded and possibly where the clutch size information will be most likely to belong. To control for confounding effects of nest type and body size, we obtained information from the Handbook of the Birds of the World online (del Hoyo et al. 2014). Nest type was classified as 1=open nest, 2=domed nest and 3=cavity nester.

To test whether clutch size was linked to the costliness of brood parasitism we included two measures of the cost of the brood parasite to each host species. First, we classified hosts according to whether or not their parasite evicts the progeny of the host (evictor vs non-evictor). Second, we quantified the size discrepancy between the parasite and the host, by dividing the size of the parasite by the size of the host, with larger parasites being more costly for smaller hosts, as applied in Medina & Langmore (2015a). Information on the parasite's body size and whether it was an evicting or non-evicting parasite was obtained from del Hoyo et al. (2014). From here on, cuckoos (subfamily Cuculinae) and honeyguides are referred to as 'evicting parasites' and cowbirds, *Clamator* cuckoos, Vidua finches, channel-billed cuckoos and Koels as 'non-evicting parasites'.

Statistical analyses

To correct for phylogenetic effects, we obtained 1000 phylogenetic trees for each dataset from the website www.birdtree.org (Jetz et al. 2012) and repeated the comparative analysis on each of these trees. We used phylogenetic generalised least squares (PGLS) regression models to account for phylogenetic relationships between species. We report the 95% highest posterior density (HPD) intervals for P-value and β coefficient for each predictor. Models were run using the Caper package in R (Orme et al. 2012), and separate models were performed for hosts of non-evicting and evicting parasites. The models included the relative size of the parasite to the host, mean latitude, body weight and nest type as explanatory variables, and mean clutch size as response variable. We ran models with and without including non-hosts, and when included, we considered non-host's costs to be zero. Latitude was converted to absolute values and thus represents distance from the equator; weight was log-transformed, given that it was non-normally distributed. We also provide information on the phylogenetic signal of each model by reporting the λ. When $\lambda = 1$ it suggests that closely-related species are evolving as expected by a Brownian motion model. Consequently, a value closer to zero would suggest that closely-related species resemble each other less than expected by a Brownian motion model (Blomberg et al. 2003). Additionally, we used the *dredge* function in the MuMIn R package (Barton 2012) to select the best model from the list of all possible models given the predictor variables, based on the AICc (corrected Akaike Information Criterion). We report which predictors were present most frequently in the best models when the analyses were repeated on each of the 1000 phylogenetic trees.

Results

Model results

Figure 2 shows a typical example of the model's output: as parasitism increases, we expect fitness to decline for host species (the non-host species, depicted by the black line in Figure 2, are of course unaffected). However, precisely how much fitness declines depends on clutch size responses and whether the host adopts one of the rejection strategies. In the example of figure 2, very low parasitism rates (the very left of the figure) predict that acceptance of the occasional parasitic egg leads to the best fitness (blue curve) among parasitized cases (all coloured curves). Here, the risks of ejecting an own egg, or abandoning an entire unparasitized nest, outweigh the occasional fitness gain through correctly dealing with a parasite. At higher parasitism rates (how high depends on the scenario), acceptance becomes outperformed by rejection behaviours.

While these results are intuitive, the central question is how the above is accompanied by evolutionary responses regarding clutch size. The circles in Figure 2 show the fitness-maximizing clutch size that underlies each fitness curve for a species that starts with a clutch size of 2 eggs. Non-rejecting individuals (strategy A, blue curve in Figure 2) have, in a limited region of a moderate parasitism rate, a lower clutch size (2 eggs) than what would be the best response if the species instead evolved rejection behaviours (3 eggs). It is nevertheless difficult to use Figure 2 to justify the tolerance hypothesis, for two reasons: there is only a very narrow range of very low parasitism rates where acceptance of all eggs yields higher fitness than either type of rejection behaviour; and more importantly, clutch sizes at no point (no value of P) evolve to be lower than that of non-hosts. Clutches are either predicted to be equally large or, at high parasitism rates, larger than those of non-hosts.

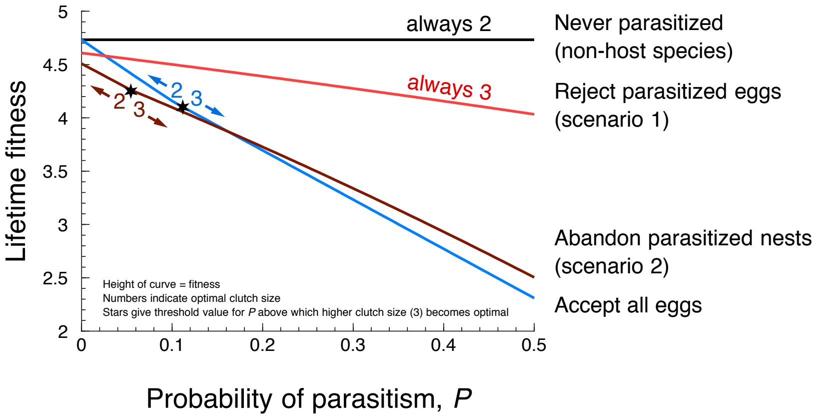


Figure 2. Fitness of non-hosts (black line), and hosts with three possible reactions to parasitism: acceptance (blue), rejection of eggs (scenario 1, bright red), or abandonment of entire nests (scenario 2, dark red), when each strategy is allowed to evolve its fitness-maximizing clutch size which is indicated as numbers along the curve: for non-hosts this is always 3, for egg-rejectors it is always 2, and for the other two scenarios the optimal clutch size changes from 2 to 3 when the rate of parasitism, P, increases (the respective threshold values are indicated with a star). The relative height of the curves indicates which behaviour performs best among the parasitized cases, and the corresponding numbers, i.e. optimal clutch sizes, indicate whether parasitism and the associated response also selects for changing the clutch size relative to the non-host clutch size (which in this example is 3 eggs). Note that there is no value of P where hosts following any strategy are selected to evolve larger clutches than non-hosts. Parameter values used in this example: $K = 25$, $k = 5$, $a = 5$, $b = 7$, $\alpha = 0.05$, $\beta = 0.2$, $C = 3$, $S = 0.7$.

The responsible factor for the increase in clutch size can be deduced by a detailed examination of a specific case, which we show, for clarity, for a particular frequency of parasitism ($P = 0.2$) and making only one of the possible contrasts in Figure 2: we now compare the non-host life history with that of hosts that are acceptors (black closed and blue open circles, respectively, in Figure 3). The parasite obviously creates a clear difference in breeding output, i.e. the number of own young fledged per breeding attempt, between hosts and non-hosts (Figure 3a). It also creates a more intriguing effect on lifespan of its hosts. Non-hosts obey a relatively steep relationship between their own clutch size and the lifespan of parents (Figure 3b, filled circles). This relationship becomes milder in hosts (Figure 3b, open circles). This is because hosts raise their own brood only some of the time; in the remaining cases the total effort is largely determined by *C*, the cost of raising a parasite, and only partially by *F*, their own clutch size decision (the cost of laying the eggs). This means that hosts' own clutch size becomes a weaker causal predictor of host lifespan. Put differently, it does not cost as much to 'try out' large clutch sizes when the related costs are, in a large fraction of breeding attempts, never paid. The weaker relationship between cost and initial clutch size (Figure 3b) then has to be weighed against the nonlinearities that are also present in the fecundity curve (Figure 3a), and the net effect can be an increase in the best clutch size (Figure 3c: fitness peaks at 2 eggs for non-hosts, and at 3 eggs for hosts).

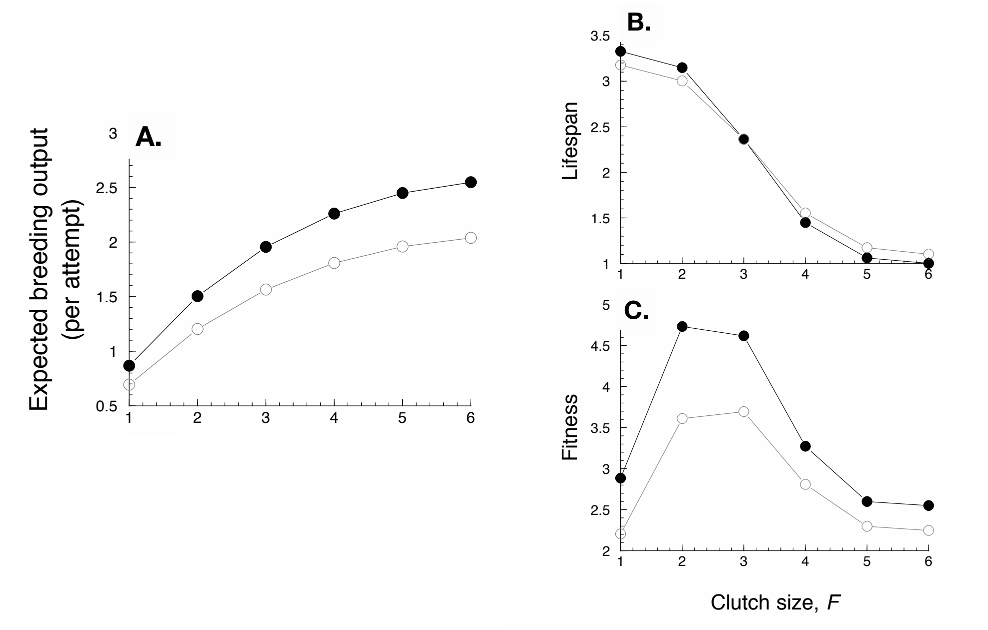


Figure 3. Fitness components (A) expected breeding output per breeding attempt, (B) expected lifespan, and (C) lifetime fitness, i.e. the product of the two components above, for two of the four strategies in Figure 2: non-hosts (in black) and hosts that are acceptors (in blue), plotted against potential clutch sizes with only the relevant ones from 1 to 6 shown for clarity. Parasitism always decreases the expected success of a single breeding attempt (A), but the effect on host lifespan (B) is more subtle: it creates a less steep relationship between the host's initial

clutch size and the parenting costs. In other words, the reduction in parental survival is less strongly dependent on clutch size in hosts than in non-hosts, because sometimes effort is spent on raising parasites (a fixed cost) rather than the whole of the own clutch (cost increasing with F). For this reason, the cost of a larger clutch can become acceptable in acceptor hosts when it would not be in non-hosts (C).

Figures 2-3 are based on specific parameter values; they do not preclude the option that the nonlinearities of the clutch size – fecundity relationship permit other cases, such as a shift towards smaller clutch sizes in some cases. We therefore explored the generality of the above argument with respect to all the parameters listed in Table S1. Because of the large number (9) of different parameters to be varied, we conducted an exhaustive search, collecting outcomes with randomly chosen parameter values in a realistic range (Table S1).

As expected in a general life history model, many parameter choices led to high reproductive effort and very low parental survival, i.e. solutions that are close to semelparity. In an avian context this can be simply interpreted as unrealistic (random choices of parameter settings that are unlikely to apply), as birds in reality are always iteroparous. Therefore we considered all parameter settings unrealistic for birds if they either (i) made parents reduce their survival by more than 95% when caring for their young (i.e. $e^{-\left(\frac{F+aF}{K}\right)^k} < 0.05$ at the fitness-maximizing F_A^*; see Supplementary Material for details), or (ii) led to optimal clutch sizes $F_A^* > 20$. We collected parameter values using the ranges specified until we had 10000 solutions that were not rejected as unrealistic as avian life histories.

Solutions were thereafter classified into six (sub)categories, separately for scenarios where rejection was done through egg rejection or nest abandonment.

A. The best option for a parasitized species is to accept parasitism rather than to evolve rejection behaviours, with three subcategories: (A+) being parasitized favours the evolution of higher clutch size than that of non-hosts, (A0) being parasitized leads to no difference in clutch size relative to that of non-hosts, (A–) being parasitized favours the evolution of smaller clutch size than that of non-hosts, when all else is equal (i.e. an otherwise identical life history).

B. The best option for a parasitized species is to evolve rejection behaviours despite this being error-prone (the frequency of errors being α and β as explained above), again with three mutually exclusive subcategories: so that being parasitized leads to (B+) higher clutch sizes, (B0) no change in clutch sizes, (B–) smaller clutch sizes.

The frequencies of classifications against each parameter are shown in Figure 4 for scenario 1 and in the supplementary Figure S1 for scenario 2. The 10000 test cases yielded outcomes in all categories, but two of the six categories occurred extremely rarely. A–, where acceptance (as opposed to rejection) behaviour evolves and this is associated with a reduction in clutch size (e.g. the tolerance hypothesis), was observed in only 16 out of 10000 cases in the scenario 1 of egg eviction (i.e. 0.16% of cases; therefore practically invisible in Figure 4). In the scenario of nest abandonment (scenario 2, Figure S1), this figure rises slightly, but only to 0.84%. Rejecting behaviour with a reduction in clutch size when parasitized (R–) was even rarer (9 and 3 cases in scenarios of egg rejection and nest abandonment, respectively; not practically visible in either Figure 4 or S1).

In the scenario where parents are able to eject particular eggs and raise the rest of the clutch, the most common categories are B0 and B+: in other words, parents evolve to reject suspicious eggs and the clutch size either stays unchanged or increases in comparison to non-hosts (Figure 4). Acceptance with no clutch size change (A0) or with an increased clutch size (A+) are also commonly found, especially if parasitism rates are low (low P), or if parents are not very well able to distinguish between their own and foreign eggs (high α and β).

In the scenario where rejecting means abandoning the entire clutch, the effects P, α and β are as in the scenario of egg rejection, but as a whole acceptance evolves more easily than in scenario 1 (figure S1: the area covered by A0 is much larger than in figure 4). This, too, makes intuitive sense: egg rejection only kills the own offspring if there is an erroneous rejection of a host egg, while nest abandonment, even if correctly targeting the parasite, always kills all the own offspring too. This means that the costs of rejection behaviours are larger in the case of nest abandonment, and the conditions for its evolution are more stringent.

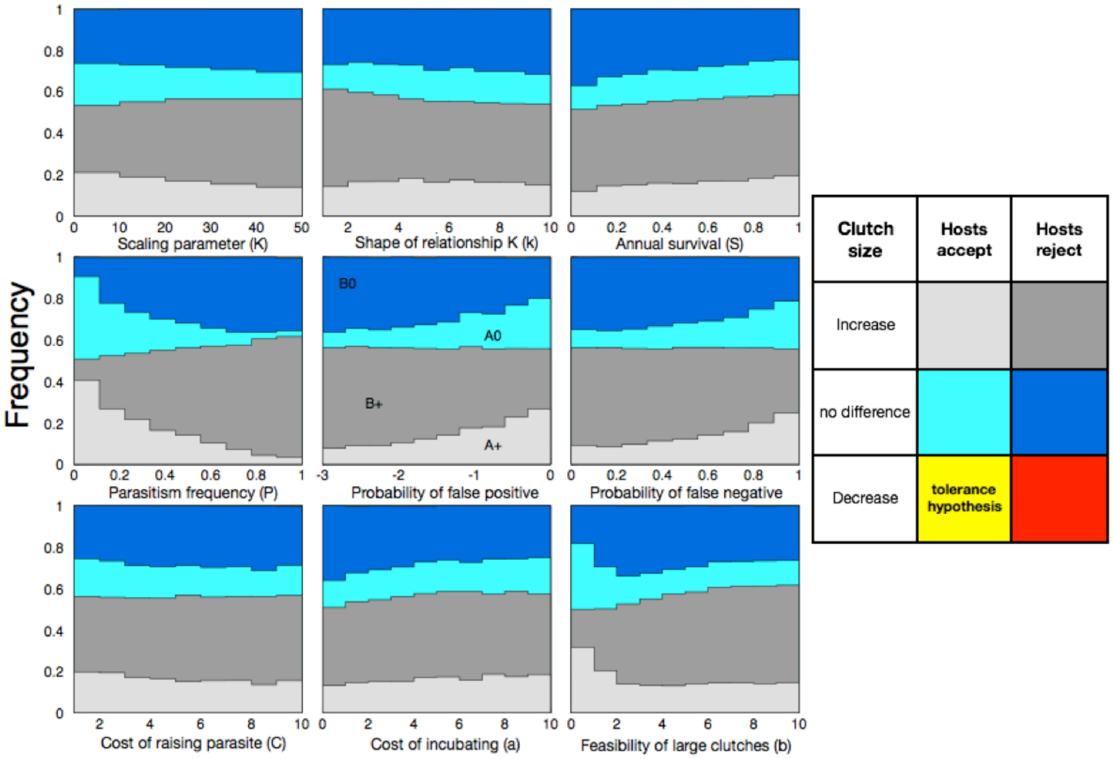


Figure 4. The 10000 collected outcomes for scenario 1 (where rejecting means ejecting eggs from a nest), with their frequency plotted against each of the 9 parameters *K*, *k*, *S*, *P*, α, β, *C*, *a*, and *b*, with colours corresponding to categories B0, A0, B+ and A+ as indicated in the central panel. Yellow and red denote A– and B–, respectively, but are practically not visible as they together form only 0.25% of all cases.

Phylogenetic comparative analysis

When analysing all the hosts and non-hosts together (with non-hosts experiencing zero costs form parasitism) both latitude and relative size were significant predictors of clutch size (PGLS, average P < 0.005), and were present in the best models. The analysis using only hosts of evicting parasites showed that latitude and the relative size of the parasite were highly significant predictors of clutch size (Table 1, Figure 5), and also present in all the best models, regardless the phylogeny used (AIC against null model > 20, Table S2). The same pattern was found if we included non-hosts in the analyses (PGLS, average P < 0.009). Hosts of costly parasites and from higher latitudes tended to have larger clutches than hosts that raise smaller, less costly parasites or occurred at lower latitudes (Figure 5). When the analysis was performed using hosts of non-evicting parasites, only latitude was a good predictor of clutch size (Table 1, AIC against null model > 4).

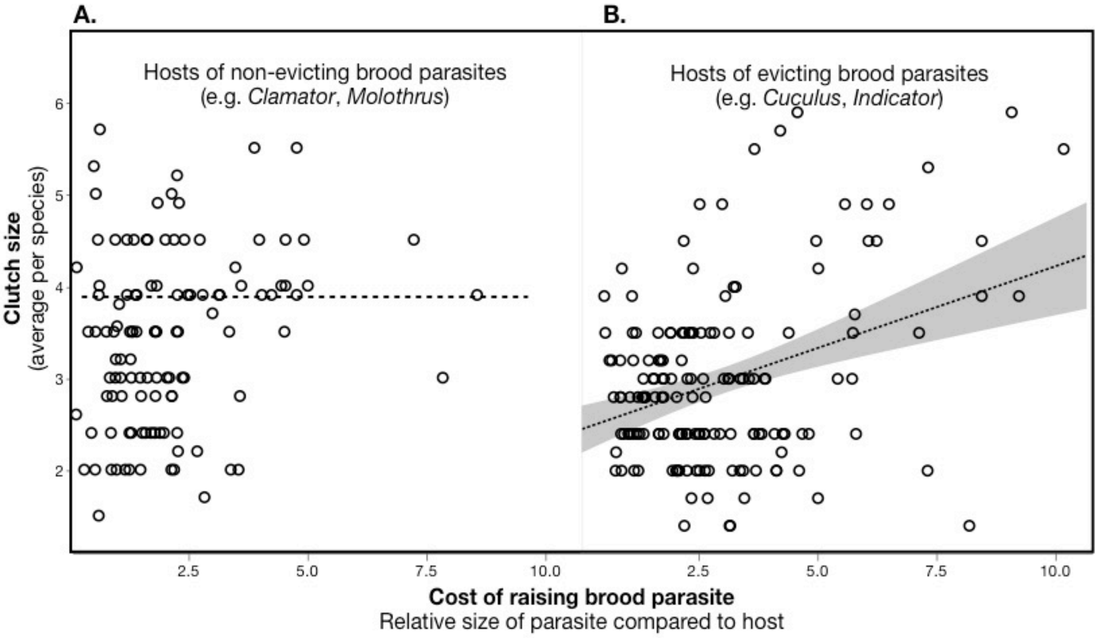


Figure 5. Association between clutch size and the costs of raising a parasite. For hosts of non-evicting parasites there is no association between the clutch size of the host and the costs of raising a parasite, but this association is significantly positive for hosts of evicting parasites.

Discussion

The evolution of a smaller clutch size has been argued to provide a potential tolerance mechanism in hosts of avian brood parasites (Brooker and Brooker 1996; Hauber 2003; Servedio and Hauber 2006). However, we suggest that the theoretical support for this rationale is poor. We modelled the evolution of clutch size in hosts of brood parasites and explored the theoretical plausibility of evolving egg acceptance and a smaller clutch size. We found that, contrary to previous suggestions, this strategy is not likely to be a common outcome. In almost no cases do hosts achieve the highest fitness by reducing their clutch size. In fact, according to our model, the best strategies for acceptor hosts should be to either show no change in clutch size, or to evolve a larger clutch size. This outcome is initially surprising, but makes sense if we consider that the presence of cuckoos in a population implies that some breeding attempts will not lead to realized fecundity, and the cost structure of those attempts differs from those of non-parasitized broods: the total parental effort is now not as strongly dependent on the host's own clutch size decisions as when there are no parasites. This makes it worthwhile to lay a large clutch in the hope of not encountering a parasite.

The size of the clutch is, of course, also impacted by other variables, such as incubation efficiency and provisioning (Lerkelund et al. 1993). Our model captures these effects through a variety of associations, relating clutch size and the consequent brood size to costs paid by parents. We also show that the evolution of rejection increases in likelihood when it is possible for the parents to eject individual eggs rather than abandon entire nests. High chances of recognition errors or low parasitism rates, in turn, lower the probability that rejection behaviours will evolve (Davies et al. 1996).

Our model obviously does not incorporate all possible effects that can occur in nature; one possibility that the model ignores is that hosts improve their ability to identify foreign eggs as a result of learning. Incorporating such an effect would alter the trade-offs by reducing the false positives and false negatives for older hosts. Calculating lifetime fitness would then become a considerably more complex task, but it appears a priori unlikely that such an exercise would drastically change the conclusions, as no value of the relevant parameters α and β lent support for clutch size reductions in the present model.

The results from the comparative analysis show an association between clutch size and the costs of raising a parasite. For hosts of evicting parasites, species that incur larger costs lay a higher number of eggs, compared to hosts that experience lower costs of parasitism or non-hosts. These results support our model, which suggests that an increase (not a decrease) in clutch size should evolve in hosts of evicting parasites, and larger clutch sizes are even more likely to evolve when the costs of parasitism increase and parasitism rates are high. There is some evidence in the literature supporting this scenario. Eurasian magpies (*Pica pica*) have higher clutch sizes in populations where parasitism rates are higher, and when they have higher egg rejection rates, which corresponds to the predictions of our model (Soler et al. 2001). Although magpies are not parasitised by evicting cuckoos, their parasite, the great spotted cuckoo (*Clamator glandarius*), is a very costly parasite, reducing to zero the reproductive outcome of their hosts in 75% of the cases (Soler et al. 2001). A similar case was reported in *Acrocephalus scirpaceus*, a host of the European cuckoo, *Cuculus canorus*, in which populations with higher parasitism and rejection rates also had higher average clutch sizes (Stokke et al. 2008). A larger clutch size might be favoured in populations that suffer extensive parasitism, where it pays to lay as many eggs as possible in the hope that the clutch is not parasitised.

An alternative possibility is that larger brood parasites are actually targeting hosts that have larger clutches, because these hosts may be better providers for the parasitic chick. This behaviour might generate the pattern we observe, where costly parasites exploit hosts with larger clutches. However, as shown in the examples above, even within host species (where costs remain similar) there may be evolution of a larger clutch size in populations with higher parasitism rates. These observations *within* species give strong support to the results from the model, and it would be interesting to study whether an increase in clutch size is observed in other host-parasite systems that vary locally in parasitism rates.

Studying tolerance mechanisms in hosts of brood parasites constitutes a very interesting avenue for future research (Medina and Langmore 2015b). However, here we show that there are neither theoretical nor empirical grounds to suggest the reduction of clutch size as a tolerance mechanism in hosts of evicting brood parasites. In fact, we show that theoretically, clutch size should increase or remain the same in response to brood parasitism pressures, and brood parasitism may affect the evolution of clutch size but in a previously overlooked direction. Our model is supported by some empirical examples (Soler et al. 2001; Cunningham and Lewis 2006; Stokke et al. 2008) and by the fact that hosts that experience the highest costs of brood parasitism also lay larger clutches. Our results contrast with previous studies suggesting clutch size reduction as a tolerance mechanism in hosts of brood parasites (Brooker and Brooker 1996; Hauber 2003), however these studies did not control for other variables known to be related to clutch size, such as latitude or body size. Furthermore, our study shows that being a host *per se* does not imply that parasitism is costly, and support the idea that the costs of parasitism vary among hosts and this has critical evolutionary consequences (Kilner 2005; Medina and Langmore 2015a). Finally, we would also like to highlight the importance of testing verbal predictions with an integrative approach.

Acknowledgements

We would like to thank the Australian National University for funding provided for I.M and the Australian Research Council for supporting NEL. We would also like to thank Thomas Wallenius, Michelle Hall and Raoul Mulder for helpful comments on previous versions of the manuscript and Sebastián Gonzalez for help with one of the figures.

References

Barton, K. 2012. MuMIn: Multi-model inference. Available: http://mumin.r-forge.r-project.org.

Blomberg, S., T. J. Garland, and A. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717-745.

Brooker, M. and L. Brooker. 1996. Acceptance by the splendid fairy-wren of parasitism by Horsfield's bronze-cuckoo: further evidence for evolutionary equilibrium in brood parasitism. Behavioral Ecology 7:395-407.

Brooker, M. G. and L. C. Brooker. 1989. Cuckoo hosts in Australia. Australian Zoological Reviews 2:1-67.

Cronin, T., M. A. Rua, and C. E. Mitchell. 2014. Why is living fast dangerous? Disentangling the roles of resistance and tolerance of disease. The American Naturalist 184:172-187.

Cunningham, E. J. and S. Lewis. 2006. Parasitism of maternal invesment selects for increased clutch size and brood reduction in a host. Behavioural Ecology 17:126-131.

Davies, N. B. 1999. Cuckoos and cowbirds versus hosts: Co-evolutionary lag and equilibrium. Ostrich: Journal of African Ornithology 70:71-79.

Davies, N. B. 2000. Cuckoos, cowbirds and other cheats. T. & A.D. Poyser, London.

Davies, N. B. 2011. Cuckoo adaptations: trickery and tuning. Journal of Zoology 284:1-14.

Davies, N. B., M. D. L. Brooke, and A. Kacelnik. 1996. Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. Proceedings of the Royal Society of London. Series B: Biological Sciences 263:925-931.

Davies, N. B. and J. A. Welbergen. 2009. Social transmission of a host defense against cuckoo parasitism. Science 324:1318-1320.

del Hoyo, J., A. Elliott, J. Sargata, D. A. Christie, and E. de Juana. 2014. Handbook of the Birds of the World Alive, Barcelona.

Feeney, W. E., I. Medina, M. Somveille, R. Heinsohn, M. L. Hall, R. A. Mulder, J. A. Stein, R. M. Kilner, and N. E. Langmore. 2013. Brood parasitism and the evolution of cooperative breeding in birds. . Science 342:1506-1508.

Forbes, S. L. and T. C. Lamey. 1996. Insurance, developmental accidents, and the risks of putting all your eggs in one basket. Journal of Theoretical Biology 180:247-256.

Friedmann, H. and L. Kiff. 1985. The parasitic cowbirds and their hosts. Western foundation of vertebrate zoology 2:225-302.

Hauber, M. and R. Kilner. 2007. Coevolution, communication, and host chick mimicry in parasitic finches: who mimics whom? Behavioral Ecology and Sociobiology 61:497-503.

Hauber, M. E. 2003. Interspecific brood parasitism and the evolution of clutch sizes. Evolutionary Ecology Research 5:559-570.

Hijmans, R. J., S. Phillips, J. Leathwick, and J. Elith. 2011. Package 'dismo'. Available online at: http://cran.r-project.org/web/packages/dismo/index.html.

Hockey, P. A. R., W. R. J. Dean, and P. G. Ryan. 2005. Roberts birds of southern Africa. The Trustees of the John Voelcker Bird Book Fund, Cape Town.

Jetz, W., C. H. Sekercioglu, and K. Bohning-gaese. 2008. The worldwide variation in avian clutch size across species and space. Plos Biology 6:e303.

Jetz, W., G. Thomas, A. Hartmann, and O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444-448.

Kilner, R. M. 2005. The evolution of virulence in brood parasites. Ornithological Science 4:55-64.

Kilner, R. M. and N. E. Langmore. 2011. Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. Biological Reviews of the Cambridge Philosophical Society 86:836-852.

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

Lerkelund, H. E., A. Moksnes, E. Roskaft, and T. H. Ringsby. 1993. An experimental test of optimal clutch size of the Fieldfare; with a discussion on why brood parasites remove eggs when they parasitize a host species. Ornis scandinavica 24:95-102.

Lyon, B. E. 1998. Optimal clutch size and conspecific brood parasitism. Nature 392:380-383.

Martin, T. E. 2015. Age-related mortality explains life history strategies of tropical and temperate songbirds. Science 349:966-970.

Martin, T. E., A. P. Møller , S. Merino, and J. Clobert. 2001. Does clutch size evolve in response to parasites and immunocompetence? Proceedings of the National Academy of Science 98:2071-2076.

Medina, I. and N. E. Langmore. 2015a. The costs of avian brood parasitism explain variation egg rejection behaviour in hosts. Biology Letters 11:1-5.

Medina, I. and N. E. Langmore. 2015b. The evolution of acceptance and tolerance in hosts of avian brood parasites. Biological Reviews 90:1-6.

Oppliger, A., P. Christe, and H. Richner 1996. Clutch size and malaria resistance. Nature 381:565.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2012. caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.

Partridge, L. and P. H. Harvey. 1985. Costs of reproduction. Nature 316:20.

Payne, R. B. 2005. The cuckoos. Oxford University Press, Oxford, UK.

Samas, P., T. Grim, M. E. Hauber, P. Cassey, K. Weidinger, and K. L. Evans. 2013. Ecological predictors of reduced avian reproductive investment in the southern hemisphere. Ecography 36:809-818.

Sato, N. J., K. Tokue, R. A. Noske, O. K. Mikami, and K. Ueda. 2010. Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. Biology Letters 6:67-69.

Servedio, M. R. and M. E. Hauber. 2006. To eject or to abandon? Life history traits of hosts and parasites interact to influence the fitness payoffs of alternative anti-parasite strategies. Journal of Evolutionary Biology 19:1585-1594.

Soler, J. J., D. Martín-Gálvez, J. G. Martínez, M. Soler, D. Canestrari, J. M. Abad-Gómez, and A. P. Møller. 2011. Evolution of tolerance by magpies to brood parasitism by great spotted cuckoos. Proceedings of the Royal Society B: Biological Sciences 278:2047-2052.

Soler, J. J., J. G. Martinez, M. Soler, and A. Moller. 2001. Life history of magpie populations sympatric or allopatric with the brood parasitic great spotted cuckoo. Ecology 82(6): 1621-1631.

Soler, M. 2014. Long-term coevolution between avian brood parasites and their hosts. Biological Reviews 89:688-704.

Spottiswoode, C. N. and M. Stevens. 2011. How to evade a coevolving brood parasite: egg discrimination versus egg variability as host defences. Proceedings of the Royal Society B: Biological Sciences 278:3566-3573.

Stoddard, M. C. and M. Stevens. 2011. Avian vision and the evolution of egg color mimicry in the common cuckoo. Evolution 65:2004-2013.

Stokke, B. G., I. Hafstad, G. Rudolfsen, A. Moksnes, A. P. Møller, E. Røskaft, and M. Soler. 2008. Predictors of resistance to brood parasitism within and among reed warbler populations. Behavioral Ecology 19:612-620.

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

Svensson, E. I. and L. Råberg. 2010. Resistance and tolerance in animal enemy-victim coevolution. Trends in Ecology & Evolution 25:267-274.

Yang, C., W. Liang, Y. Cai, S. Shi, F. Takasu, A. P. Møller, A. Antonov, F. Fossøy, A. Moksnes, E. Røskaft, and B. G. Stokke. 2010. Coevolution in Action: Disruptive Selection on Egg Colour in an Avian Brood Parasite and Its Host. PLoS ONE 5:e10816.

Tables

		Evicting parasites			**Non-evicting parasites**	
Predictor	**estimate**	**p**	λ	**estimate**	**p**	λ
Cost	0.198 to 0.241	**Always < 0.0001**	0.91 to 1	-0.205 to 0.051	0.68 to 0.999	0.849 to 0.975
Latitude	0.197 to 0.272	**Always < 0.0001**	0.91 to 1	0.014 to 0.022	**0.002 to 0.038**	0.849 to 0.975
Weight	0.502 to 1.311	0.003 to 0.211	0.91 to 1	-1.113 to -0.497	0.127 to 0.487	0.849 to 0.975
Nest type	-0.70 to -0.31	0.005 to 0.093	0.91 to 1	-0.482 to -0.234	0.551 to 0.884	0.849 to 0.975
Nest type	-0.053 to 0.493	0.446 to 0.979	0.91 to 1	-0.309 to -0.074	0.372 to 0.539	0.849 to 0.975
Nest type	0.160 to 0.372	0.673 to 0.860	0.91 to 1	0.345 to 0.469	0.688 to 0.999	0.849 to 0.975

Table 1. Statistics for each parameter for each predictor of host clutch size, for hosts of evicting parasites, such as most cuckoos and honeyguides and non-evicting parasites. We report the 95% HPD intervals across 1000 phylogenetic trees, for each parameter. Significant values are highlighted in bold.

Supplementary material

File including model details, supplementary table with model parameters T1 and supplementary graphs S1 and S2.

The model

The details of the model are as follows. A mother lays F_A eggs if it follows the A strategy, and F_R eggs if it follows the R strategy. In case of the bird being a host species, each breeding attempt is parasitized with probability P; non-hosts are all assumed to be A-strategists with P set to zero. The brood parasite is assumed to remove one host egg at laying, and all host eggs or chicks at hatching (the latter requires that the host has not detected the foreign egg). Strategy R hosts do not reject all of their parasitized eggs (in scenario 1) or nests (in scenario 2), as rejection behaviour is associated with making errors based on false positives — abandoning a nest or ejecting an egg that contains no parasite — and false negatives — raising a parasite. We denote these error probabilities with α and β, respectively. This means that hosts that raise parasites can be either A birds, or R birds that have made a false negative judgement (probability β). If a parasite is allowed to stay in the nest, it is assumed to destroy all of the parent's own young.

Scenario 2 is simpler than scenario 1, as it allows only two options: a bird either abandons a nest or will attempt to raise all the young in the nest, with the latter possibility including the case where the parasite is the only individual remaining in the nest. In scenario 1, the possibilities are more complex: if there is a parasite that is not ejected then the nest will have one parasite and no own young. If there is a parasite that is ejected, then the clutch has first been reduced from F to F–1 (the parasite removing one host egg which we assume is not replaced), after which there is still a false positive error rate α that targets the host's own eggs. We therefore need to specify a binomially distributed random variable $N_1 \sim B(F-1, 1-\alpha)$ for the number of own eggs that survive this stage (B denotes the binomial distribution with parameter $1-\alpha$).

Finally, if the nest was not parasitized at all, the same logic without the reduction to $F-1$ leads to a binomially distributed random variable $N_0 \sim B(F,1-\alpha)$.

We next specify how breeding success is formed. Because it is not realistic to assume that all eggs that a parent attempts to raise lead to fledged young, we assume (for either scenario) that an unparasitized nest with F eggs will fledge $Fe^{-F/b}$ young on average; if the brood is first reduced from F to N surviving eggs (scenario 2), this becomes $Ne^{-N/b}$. The parameter b describes the feasibility of large clutch sizes, and it reflects constraints of incubation and parenting efficiency: clutch sizes larger than b become counterproductive as they lead to fewer young fledging than if the original clutch contained only b young.

A host is thus expected to evolve clutch sizes in the range $0 < F \leq b$. The best value of F depends also on costs of laying the F eggs as well as caring for nestlings. In a system that includes brood parasites the young that can be either own, or parasitic. This makes it important to differentiate between laying costs (a host pays these costs for its own eggs but not the parasite's) and all other forms of parenting effort. We therefore assume that the total effort the host spends caring for a brood that started with a clutch size F but ends up having N nestlings and a parasite load of C is $F+a(N+C)$. Here the 'parasite load' C refers to the relative costs, in equivalents of nestlings, of raising one parasite; if, for example, one parasite requires as much effort to raise as 5 own nestlings, then $C = 5$. The parameter a reflects the cost of incubating and feeding the young relative to laying the eggs: if a is large ($a >> 1$), then incubating and feeding effort has a larger detrimental impact on host survival than the effort spent on laying the clutch. Note that our assumptions also mean that we don't have simultaneously $N > 0$ and $C > 0$; for example, a scenario 1 host that starts with 5 young, is parasitized, succeeds in removing the parasite, but also erroneously ejects one of its own eggs will spend effort $F+a(N+C)$ where $F = 5$, $N = 4$ and $C = 0$.

To complete the model, we also need to translate parental effort into the relevant life-history currency: its consequences for host survival. Effort is assumed to have a negative effect on survival, but the shape could take many forms, reflecting variations in the ability of hosts to take care of small or large broods. We consequently use two parameters, *K* and *k* (analogous to *λ* and *k* as parameters of the Weibull distribution), to describe exactly how increasing parental effort impacts host survival: survival is assumed to be $Se^{-\left(\frac{F}{K}\right)^k}$ if the brood was abandoned (the only effort was *F*, the minimum that was spent laying the eggs), but it drops to $Se^{-\left(\frac{F+a(N+C)}{K}\right)^k}$ if the host raised the brood that contains own nestlings or parasites (so that either $N > 0$ or $C > 0$). *S* is the survival in the absence of any parenting effort. The parameter *K* scales the sensitivity of survival to parenting effort by describing the amount of effort that leads to survival being reduced to a factor e^{-1} = 0.368 of what could be achieved if no effort had been spent. The parameter *k* describes the shape of the relationship: if *k* is large, then survival remains relatively intact up to efforts close to *K*. If *k* is small, then even small clutches are sufficient to cause great reductions in host survival.

A host's lifetime reproductive success depends on the expected fecundity per breeding attempt as well as lifespan (which takes the form 1/(1–*S*) if annual survival equals *S*; this general form gets modified according to parental effort for each of the cases below). A host using strategy A, i.e. accept all broods, has an expected output of (1–*P*) $Fe^{-F/b}$ per breeding attempt, and a lifespan $\left(1-\left(PSe^{-\left(\frac{F+aC}{K}\right)^k}+(1-P)Se^{-\left(\frac{F+aF}{K}\right)^k}\right)\right)^{-1}$, thus the lifetime reproductive output to be maximized (with respect to *F*) is

$$w_A = \frac{(1-P)\,Fe^{-\frac{F}{b}}}{1-\left(PSe^{-\left(\frac{F+aC}{K}\right)^k}+(1-P)Se^{-\left(\frac{F+aF}{K}\right)^k}\right)}.$$

Non-host species use the same equation, with *P* set to zero.

The fitness of a strategy R host is somewhat more complicated, because rejection behaviour associates with false positives and false negatives (parameters *α* and β), and because we have two different scenarios to deal with (scenarios 1 and 2). We first derive the equations for scenario 2, as it is the simpler case. In this scenario, a nest may be parasitized (probability *P*) and the host pays the cost of raising the parasite (effort

$F{+}aC$) because of a false negative (probability β); or the nest is parasitized (probability P) and rejected (probability 1–β), which reduces the effort to what was spent laying the eggs, F; or the nest is unparasitized (probability 1–P), in which case a false positive (α) can happen leading to effort F and no current reproductive effort, or, finally, there is no false positive (1–α) and the nest yields an output $Fe^{-F/b}$, and host survival is now based on effort $F{+}aF$. Combining all the options leads to expected breeding output as indicated in the numerator and lifespan-impacting factors in the denominator of fitness

$$w_{\mathrm{R2}} = \frac{(1\text{–}\,P)\,(1-\alpha)Fe^{-\frac{F}{b}}}{1-\left(PS\left((1-\beta)e^{-\left(\frac{F}{K}\right)^{k}}+\beta e^{-\left(\frac{F+aC}{K}\right)^{k}}\right)+(1-P)S\left(\alpha e^{-\left(\frac{F}{K}\right)^{k}}+(1-\alpha)e^{-\left(\frac{F+aF}{K}\right)^{k}}\right)\right)}$$

for rejectors of scenario 2.

For scenario 1, we need to use binomial probabilities to translate between F and N, so that expected breeding output from one attempt becomes $(1\text{–}\,P\beta)\ \sum_{N=0}^{F}\binom{F}{N}(1-\alpha)^{N}\alpha^{F-N}\,Ne^{-\frac{N}{b}}$; and this together with expected lifespan calculations (which also become impacted by binomial probabilities of each brood size and the consequent effort) leads to fitness

$$w_{\mathrm{R1}} = \frac{(1\text{–}\,P\beta)\ \sum_{N=0}^{F}\binom{F}{N}(1-\alpha)^{N}\alpha^{F-N}\,Ne^{-\frac{N}{b}}}{1-\left(P\beta Se^{-\left(\frac{F+aC}{K}\right)^{k}}+(1-P\beta)S\sum_{N=0}^{F}\binom{F}{N}(1-\alpha)^{N}\alpha^{F-N}\,e^{-\left(\frac{F+aN}{K}\right)^{k}}\right)}$$

for rejectors of scenario 1.

Parameter	Interpretation	Range values
A	Host accepts eggs	–
R	Host rejects eggs or nests	–
Scenario 1	Rejection through egg eviction	–
Scenario 2	Rejection through nest abandonment	–
P	Parasitism rate	[0, 1]
α	Probability of false positive judgment; host rejects when no parasite	Logarithmically distributed between 10^{-3} and 1
β	Probability of false negative judgment; host raises a parasite	[0, 1]
a	Costs of incubating and feeding relative to egg-laying	[0, 10]
b	Feasibility of large clutches	[0, 10]
C	Costs of raising a parasite relative to raising own young	[1, 10]
K	Scaling of the parental effort level with respect to parental survival reductions	[1, 50]
k	Shape of the relationship between K and parental survival	[1, 10]
S	Annual survival after zero parental effort	[0, 1]
W	Expected lifetime reproductive output	Evolves
F	Clutch size (best clutch sizes denoted F_A^* and F_R^*)	Evolves (to a value between 1 and b)
N	Number of own eggs that survive	Computed from F and other parameters

Table T1. Notation used in the model; the range of parameters refers to values used to collect 10000 examples per scenario, with a uniform distribution within the range unless otherwise specified.

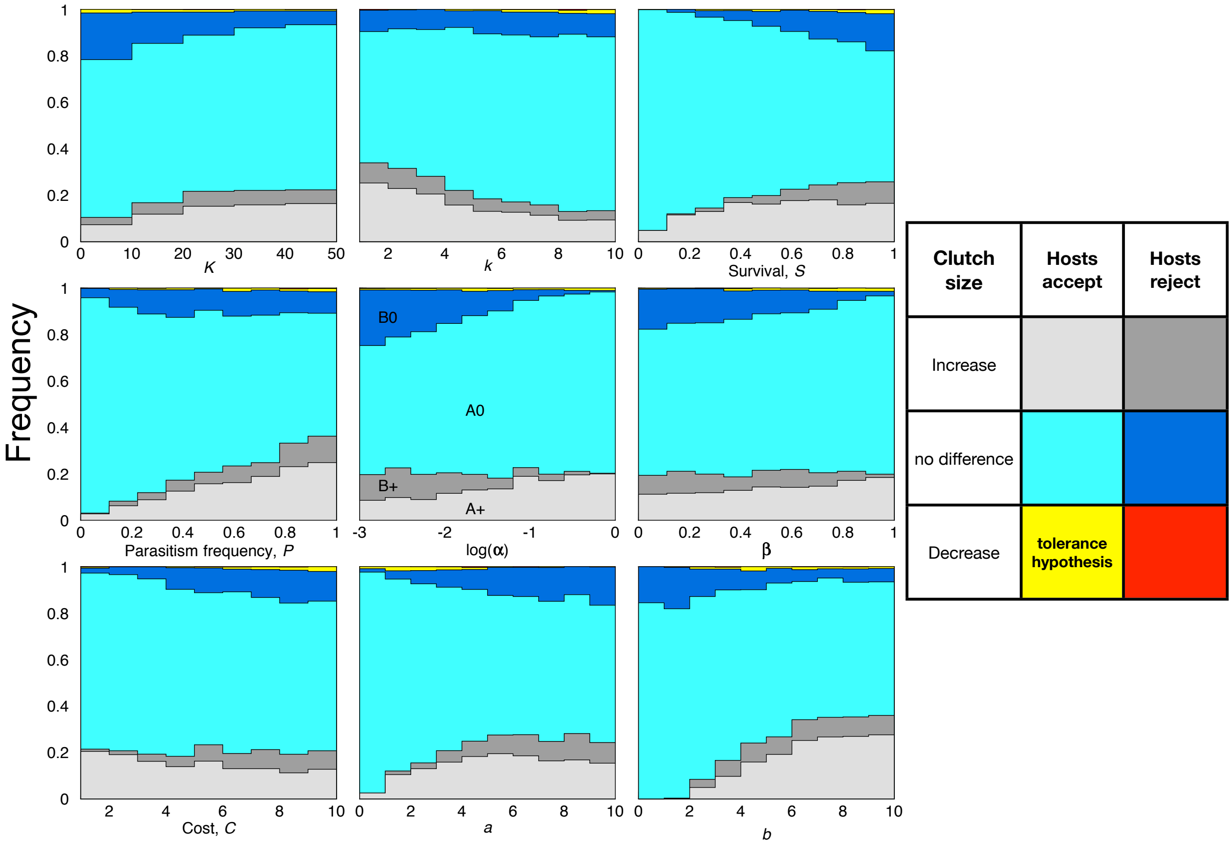


Figure S1. The 10000 collected outcomes for scenario 2 (where rejecting means abandoning the entire nest), with outcome classification frequency plotted against each of the 9 parameters *K*, *k*, *S*, *P*, α, β, *C*, *a*, and *b*, with colours corresponding to categories B0, A0, B+ and A+ as indicated in the central panel. Yellow and red denote A– and B–, respectively, but red is practically not visible.

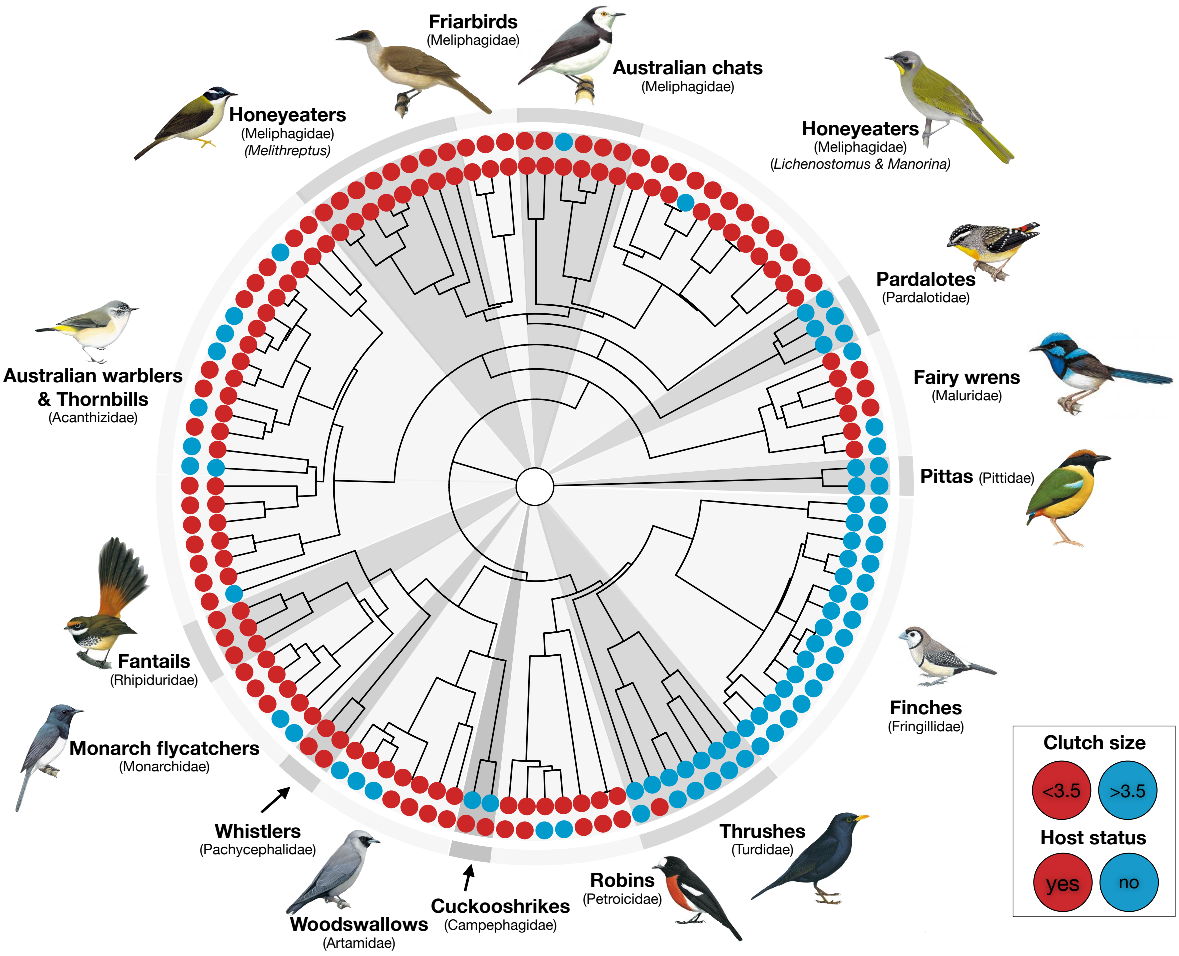


Figure S2. Phylogenetic tree of Australian hosts (red, inner circle) and non-hosts (blue, outer circle). Most of the non-host species are finches and many of these are cavity nesters, with usually larger clutches.

Chapter 3

The costs of avian brood parasitism explain variation in egg rejection behaviour in hosts

Published in *Biology Letters* (2015)

BIOLOGY
LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Medina I, Langmore NE. 2015 The costs of avian brood parasitism explain variation in egg rejection behaviour in hosts. *Biol. Lett.* **11**: 20150296.
http://dx.doi.org/10.1098/rsbl.2015.0296

Received: 13 April 2015
Accepted: 11 June 2015

Subject Areas:
behaviour, evolution, ecology

Keywords:
brood parasitism, egg rejection, defence costs

Author for correspondence:
Iliana Medina
e-mail: iliana.medina@anu.edu.au

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2015.0296 or via http://rsbl.royalsocietypublishing.org.

THE ROYAL SOCIETY
PUBLISHING

Animal behaviour

The costs of avian brood parasitism explain variation in egg rejection behaviour in hosts

Iliana Medina and Naomi E. Langmore

Department of Ecology, Evolution and Genetics, Australian National University, Canberra, Australian Capital Territory, Australia

Many bird species can reject foreign eggs from their nests. This behaviour is thought to have evolved in response to brood parasites, birds that lay their eggs in the nest of other species. However, not all hosts of brood parasites evict parasitic eggs. In this study, we collate data from egg rejection experiments on 198 species, and perform comparative analyses to understand the conditions under which egg rejection evolves. We found evidence, we believe for the first time in a large-scale comparative analysis, that (i) non-current host species have rejection rates as high as current hosts, (ii) egg rejection is more likely to evolve when the parasite is relatively large compared with its host and (iii) egg rejection is more likely to evolve when the parasite chick evicts all the host eggs from the nest, such as in cuckoos. Our results suggest that the interactions between brood parasites and their hosts have driven the evolution of egg rejection and that variation in the costs inflicted by parasites is fundamental to explaining why only some host species evolve egg rejection.

1. Introduction

Avian brood parasites lay their eggs in the nest of other species, their hosts, who raise a foreign chick [1]. Brood parasitism is costly to the hosts and has been proposed as the driver of the evolution of defences [1], such as attacking parasites [2] or rejecting their chicks or fledglings [3,4]. One of the most intensively studied defences is egg rejection. To date, egg rejection has been studied in around 200 different bird species [5,6]. Surprisingly, despite the effectiveness of egg rejection as a defence against brood parasitism, not all host species reject foreign eggs [5]. There is still no consensus about which particular traits are associated with the evolution of egg rejection [6–8].

Previous studies suggest that several factors may explain variation in egg rejection behaviour. First, the most obvious variable is whether a species is a suitable and a current host of a brood parasite [6]. Suitability is assessed based on the diet of the host chicks and nesting habits [1]. Second, brood parasites vary in the costs they inflict on host fecundity (termed virulence *sensu* Kilner [9]). Some parasites such as the common cuckoo (*Cuculus canorus*) inflict very high costs, because the newly hatched parasite evicts all the host's eggs from the nest (evicting parasites). Conversely, hosts of non-evicting brood parasites (such as the parasitic cowbirds, *Molothrus* spp.) may succeed in rearing their own progeny alongside the parasite chick [1]. Third, the costs of parasitism can vary between host species. For instance, small hosts that rear large parasites will possibly suffer higher provisioning costs than host species that are of similar size to their parasites [10]. Similarly, hosts (of evicting parasites) that lay large clutches will possibly carry larger costs if their whole brood is lost than hosts with small clutches, so selection for egg rejection should be stronger in the former. Fourth, nest type may be an important predictor of egg rejection, given that poor visibility inside enclosed nests may constrain a host's ability to discriminate between eggs [6].

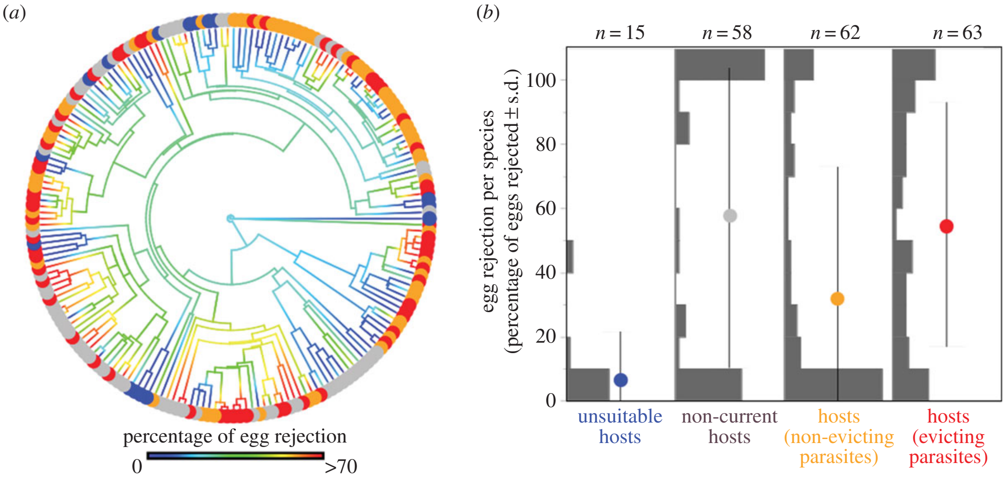


Figure 1. (*a*) Random possible phylogeny of the 198 species used in the study with branch colours indicating the percentage of egg rejection for the species. Circles at the tip of the branches indicate the host status of each species, and the colour legend is the same as in figure 1*b*. There is significant phylogenetic signal in both host status and percentage of rejection. (*b*) Histogram with mean and standard deviations of egg rejection percentage for each category of host status. Egg rejection rates of unsuitable hosts (blue) and hosts of non-evicting parasites (orange) differed significantly from those of non-current hosts (grey, $p = 0.019$) and hosts of evicting parasites (red, $p = 0.005$).

Finally, latitude may also be related to egg rejection. Breeding seasons are shorter at high latitudes, which may reduce opportunities for re-nesting following parasitism and lead to stronger selection for egg rejection [6,11].

Here, we compile data on egg rejection rates from 198 avian species and perform a series of comparative analyses to test which of the variables mentioned above can explain the evolution of egg rejection. By using a much larger dataset and recent, more robust phylogenetic techniques and phylogenies than previous studies [6–8,11], we aimed to arrive at a unified understanding of why egg rejection evolves in birds.

2. Methods

(a) Data collection

We obtained egg rejection data for 198 species from published studies (see electronic supplementary material, table S1). We classified host status of species into one of four categories based on data published in the original sources; suitability was based on dietary requirements or nesting habits: (1) unsuitable hosts ($n = 15$), (2) suitable hosts but have no known reports of parasitism (non-current hosts hereafter, $n = 58$), (3) hosts of parasites that do not evict host eggs ($n = 62$) and (4) hosts of parasites that evict all host eggs ($n = 63$). For known hosts (categories 3 and 4), we also obtained information on whether it was a rare or common host from the original article. We collected information on average clutch size, host nest type, and parasite and host body size from [12] or [13]. Latitudinal distribution was obtained using the function *gbif* in the DISMO R package [14] (see the electronic supplementary material).

(b) Phylogenetic analyses

To account for phylogenetic relatedness between species, we ran all analyses in 1000 possible phylogenetic trees obtained from www.birdtree.org [15]. To test which variables were the best predictors of egg rejection we ran phylogenetic regressions using the function PGLS in the R package CAPER [16]; in our models, the response variable was percentage of egg rejection per species, and the predictor variables were host status, clutch size, host nest type and host/parasite body size (details in the electronic supplementary material). We report the β estimate and p for each predictor and the λ for the whole model. We also calculated phylogenetic signal on the rate of egg rejection and on host status by using Pagel's λ in the PHYTOOLS package in R [17]. Higher (closer to 1) and significant values of λ suggest that the distribution of the traits (or the relationships) are linked with the structure of the phylogeny.

3. Results

Host status and egg rejection had a significant phylogenetic signal (host status $\lambda = 0.81$, $p < 0.001$, egg rejection $\lambda = 0.91$, $p < 0.001$, figure 1*a*). Across all species, egg rejection was predicted by latitude and host status (table 1 and figure 1*b*). There were higher rejection rates at higher latitudes, and unsuitable hosts had significantly lower egg rejection rates than all the other groups. Non-current hosts had higher rejection rates than hosts of non-evicting parasites ($\beta = -18.34 \pm 0.79, p = 0.019 \pm 0.0054$) and similar rejection rates to hosts of evicting parasites ($\beta = -3.22 \pm 0.73$, $p = 0.39 \pm 0.024$). Clutch size and nest type did not have any influence on egg rejection rates ($\beta = -5.49 \pm 0.181$, $p = 0.074 \pm 0.011$ and $\beta = 2.53 \pm 0.79$, $p = 0.72 \pm 0.077$, $\lambda = 0.88 \pm 0.14$). For hosts of non-evicting parasites, all predictor variables were non-significant ($p > 0.3$). For hosts of evicting parasites, the cost of raising a parasitic chick (parasite mass/host mass) was the only significant predictor of egg rejection (table 1 and figure 2), where hosts that are exploited by relatively larger parasites show higher egg rejection. There were no significant differences in egg rejection between common and rare hosts ($\beta = 4.51 \pm 0.47$, $p = 0.65 \pm 0.02$).

4. Discussion

The best predictor of egg rejection was host status, supporting the argument that egg rejection has evolved as a defence against brood parasitism [6]. Additionally, we found significant phylogenetic signal in host status and egg rejection, suggesting that

Table 1. Final models including only significant predictors, we report the standard deviation in 1000 phylogenetic trees for each statistic.

model and predictors	β estimate	p-value	λ whole model
all species			0.88 ± 0.14
host status	40.02 ± 1.021	0.0012 ± 0.0003	
latitude	0.4032 ± 0.01	0.0008 ± 0.0002	
hosts of evicting parasites			0.76 ± 0.14
cuckoo mass/host mass	4.05 ± 0.13	0.006 ± 0.001	

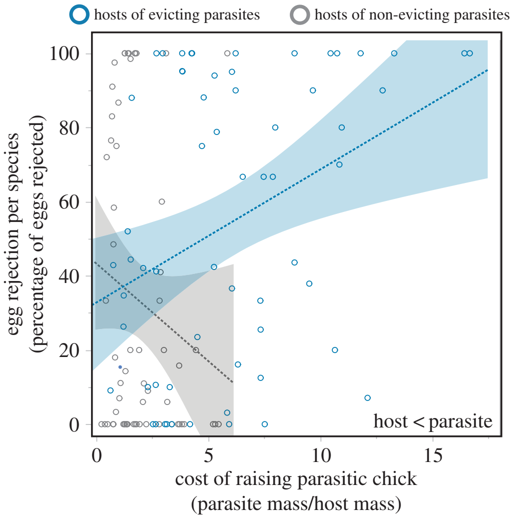


Figure 2. For hosts of evicting parasites such as cuckoos (blue), egg rejection rates increase with increasing relative size of the parasite to the host ($\beta = 4.05$, $p = 0.006$). This relationship is not significant for hosts of non-evicting parasites (grey, $p = 0.15$), which are usually more similar in size to the parasite.

phylogenetic relatedness can predict whether a species is a host and a rejecter. This is not surprising, given that hosts are suitable based on traits like diet or nesting habits, which are very likely to be phylogenetically conserved [18,19].

Interestingly, we found that non-current, but suitable host species presented egg rejection rates (mean = 57.99%) that were similar to those of hosts of evicting parasites (mean = 59.03%), suggesting that non-current, suitable hosts may have been hosts in the past. Rothstein [20] proposed that egg rejection carries insignificant costs and may persist as a relic behaviour for years in the absence of brood parasitism, and this has been supported by empirical studies [21] and is discussed in detail in a recent review [5]. Further, our findings suggest that brood parasites switch to a new host when egg rejection becomes a highly reliable defence in their current host, since rejection rates of 100% were more common in non-current hosts (49% of the species) than hosts (21%). These results provide support for both the single trajectory model of brood parasite–host interactions [20], and Soler's [5] model of a cumulative increase in the number of species with successful resistance; hosts evolve highly effective egg rejection and retain it, leading to a host switch by the parasite.

Brood parasite species differ in the costs they inflict on their hosts; some parasite chicks kill or evict all host young, whereas others tolerate them [9]. As predicted, egg rejection was more likely in hosts of evicting than in hosts of non-evicting parasites. This suggests that there is weaker selection on hosts of non-evicting parasites for the evolution of defences. However, an alternative explanation is the duration of the brood parasite–host association; cuckoos are a much older lineage (approx. 60 Ma) than cowbirds (approx. 3 Ma) and this may also explain why more of their hosts have evolved egg rejection [22]. Also, cowbird species tend to exploit a wider variety of hosts than other brood parasites, and this may lead to lower parasitism rates of individual host species and thereby diffuse the intensity of the interactions with their hosts [22].

The evolution of egg rejection in hosts of evicting parasites (59.03%) and non-evicting parasites (32.84%) appears to be influenced by different variables. In hosts of evicting parasites, the relative size of the parasite and its host can determine whether a species is a rejecter or not. When the host is very small relative to the parasite, it is more likely to evolve egg rejection. Interestingly, this trend was not found for hosts of non-evicting parasites, which supports the idea that costs are higher for hosts of evicting parasites. Our results also confirm that hosts and parasites are more similar in size in the cowbird–host system than in the cuckoo–host system, as has been noted previously [10]. Additionally, we found that latitude is a good predictor of egg rejection behaviour; species at higher latitudes showed higher egg rejection rates. Latitude is highly correlated with seasonality [12]; thus, the pattern detected suggests that egg rejection is more likely to evolve in highly seasonal environments, where opportunities for re-nesting are more scarce, and the costs of being parasitized may be higher [6]. Clutch size and nest type were not predictors of egg rejection in hosts of evicting or non-evicting parasites.

In conclusion, we found support for the idea that non-current hosts can retain egg rejection, which may be a cost-free behaviour, and that the likelihood of evolving egg rejection is influenced by the costs of parasitism; egg rejection is more likely to evolve if all host offspring are lost as a result of parasitism, if the parasite is large relative to the host, and if opportunities for re-nesting following parasitism are low.

Data accessibility. The dataset supporting this article has been uploaded as part of the electronic supplementary material.

Authors' contributions. I.M. and N.E.L conceived the study, collected the data and wrote the paper. I.M. performed the data analysis.

Competing interests. We declare we have no competing interests.

Funding. N.E.L. was funded by the Australian Research Council, DP110101966.

Acknowledgements. We thank Nick Davies for help acquiring some of the data, and two anonymous reviewers for very helpful suggestions on the manuscript.

References

1. Davies NB. 2000 *Cuckoos, cowbirds and other cheats*. London, UK: T & AD Poyser.
2. Feeney WE, Welbergen JA, Langmore NE. 2012 The front-line of avian brood parasite–host coevolution. *Anim. Behav.* **84**, 3–12. (doi:10.1016/j.anbehav.2012.04.011)
3. Langmore NE, Stevens M, Maurer G, Heinsohn R, Hall ML, Peters A, Kilner RM. 2011 Visual mimicry of host nestlings by cuckoos. *Proc. R. Soc. B* **278**, 2455–2463. (doi:10.1098/rspb.2010.2391)
4. de Mársico MC, Gantchoff MG, Reboreda J. 2012 Host–parasite coevolution beyond the nestling stage? Mimicry of host fledglings by the specialist screaming cowbird. *Proc. R. Soc. B* **279**, 3401–3408. (doi:10.1098/rspb.2012.0612)
5. Soler M. 2014 Long-term coevolution between avian brood parasites and their hosts. *Biol. Rev.* **89**, 688–704. (doi:10.1111/brv.12075)
6. Langmore NE *et al*. 2005 The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behav. Ecol.* **6**, 686–692. (doi:10.1093/beheco/ari041)
7. Soler JJ. 1998 Do life-history variables of European cuckoo hosts explain their egg-rejection behavior? *Behav. Ecol.* **10**, 1–6. (doi:10.1093/beheco/10.1.1)
8. Peer BD, Sealy SG. 2004 Correlates of egg rejection in hosts of the brown-headed cowbird. *Condor* **106**, 580–599. (doi:10.1650/7412)
9. Kilner RM. 2005 The evolution of virulence in brood parasites. *Ornithol. Sci.* **4**, 55–64. (doi:10.2326/osj.4.55)
10. Krüger O, Davies NB. 2004 The evolution of egg size in the brood parasitic cuckoos. *Behav. Ecol.* **15**, 210–218. (doi:10.1093/beheco/arg104)
11. Brooker M, Brooker L. 1996 Acceptance by the splendid fairy-wren of parasitism by Horsfield's bronze-cuckoo: further evidence for evolutionary equilibrium in brood parasitism. *Behav. Ecol.* **7**, 395–407. (doi:10.1093/beheco/7.4.395)
12. Jetz W, Sekercioglu CH, Bohning-gaese K. 2008 The worldwide variation in avian clutch size across species and space. *PLoS Biol.* **6**, e303. (doi:10.1371/journal.pbio.0060303)
13. del Hoyo J, Elliott A, Sargata J, Christie DA, de Juana E. 2014 *Handbook of the birds of the world alive*. Barcelona, Spain: Lynx Editions.
14. Hijmans RJ, Phillips S, Leathwick J, Elith J. 2011 *Package 'dismo'*. See http://cran.r-project.org/web/packages/dismo/index.html (accessed January 2015).
15. Jetz W, Thomas G, Hartmann A, Mooers O. 2012 The global diversity of birds in space and time. *Nature* **491**, 444–448. (doi:10.1038/nature11631)
16. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012 *caper. Comparative analyses of phylogenetics and evolution in R*, version 0.5. See http://caper.r-forge.r-project.org/ (accessed January 2015).
17. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)
18. Zyskowski K, Prum RO. 1999 Phylogenetic analysis of the nest architecture of neotropical ovenbirds (Furnaidae). *Auk* **116**, 891–911. (doi:10.2307/4089670)
19. Brumfield RT, Tello JG, Cheviron ZA, Carling MD, Crochet N, Rosenberg KV. 2007 Phylogenetic conservatism and antiquity of a tropical specialization: army-ant-following in the typical antbirds (Thamnophilidae). *Mol. Phylogenet. Evol.* **45**, 1–13. (doi:10.1016/j.ympev.2007.07.019)
20. Rothstein SI. 2001 Relic behaviours, coevolution and the retention versus loss of host defences after episodes of avian brood parasitism. *Anim. Behav.* **61**, 95–107. (doi:10.1006/anbe.2000.1570)
21. Peer BD, Kuehn MJ, Rothstein SI, Fleischer RC. 2011 Persistence of host defence behaviour in the absence of avian brood parasitism. *Biol. Lett.* **7**, 670–673. (doi:10.1098/rsbl.2011.0268)
22. Winfree R. 1999 Cuckoos, cowbirds and the persistence of brood parasitism. *Trends Ecol. Evol.* **14**, 338–343. (doi:10.1016/S0169-5347(99)01643-2)

SUPPLEMENTARY MATERIAL

We obtained egg rejection information for 194 species from Soler [6] and a number of additional references (supplementary material Table 1). Our dataset did not include experiments where nests where abandoned. We reclassified host status for each species into one of four different categories; 1) species that are unsuitable hosts because of their dietary requirements or nesting habits (n=15), 2) species that are suitable hosts but currently have no reports of parasitism (n=58), 3) hosts parasitized by species that don't evict host eggs (n=58) and 4) hosts parasitized by species that evict all host eggs (n=63). This information was obtained directly from the paper where the original experiment was published. Within host species, we also obtained information on whether it was a rare or common host. We collected information on average clutch size per species from [1], or the Handbook of Birds of the World Alive [2]. Information on parasite virulence, host nest type and parasite and host body size was obtained from the Handbook of the Birds of the World Alive [2]. Latitudinal distribution was obtained by calculating the average latitude from 2000 random records from the GBIF database, using the function *gbif* in the dismo R package [3].

Phylogenetic analyses

In the model that included all species the possible predictors for egg rejection were: host status (from 1 to 4), nest type (open or closed), clutch size and latitude. To test which were the best predictors of egg rejection within hosts of evicting or non-evicting parasites, we used the same predictors as the previous model, plus a measure of the costs of raising a cuckoo, which was derived from dividing the mass of the cuckoo by the mass of it's host. We report the β estimate and P for each predictor and the λ for the whole model. To confirm our results, we also compared competing models using the values of the Akaike's Information Criterion (AIC) by using the *dredge* function of the MuMIn R package [4] models that had an AICc difference of less than two were considered to be equally good [4]. Results were qualitatively the same for both model selection approaches, so we only report results on the first methodology. Since all variables were included in the same model, the results shown take into account possible correlations between predictor variables. We also calculated phylogenetic signal on the rate of egg rejection and on host status by using Pagel's λ in the *phytools* package in R [5]. Higher (closer to 1) and significant values of λ suggest that the distribution of the traits (or the relations in the model) are linked with the structure of the phylogeny.

Results from AIC model comparison

Across all species, egg rejection was predicted by latitude and host status and the model with these two variables had the lowest AICc (average AICc= 1924). For hosts of non-evicting parasites, latitude was a marginally significant predictor of egg rejection and the best model included only this variable (average AICc= 588.1). For hosts of evicting parasites, the cost of raising a parasitic chick (parasite mass / host mass) was the only significant predictor of egg rejection and it was the only variable in the best model (average AICc=588.4).

References

[1] Jetz, W., Sekercioglu, C.H. & Bohning-gaese, K. 2008 The worldwide variation in avian clutch size across species and space. *Plos Biology* **6**, e303.

[2] del Hoyo, J., Elliott, A., Sargata, J., Christie, D.A. & de Juana, E. 2014 *Handbook of the Birds of the World Alive*. Lynx Editions ed. Barcelona.

[3] Hijmans, R.J., Phillips, S., Leathwick, J. & Elith, J. 2011 Package 'dismo'. Available online at: http://cran.r-project.org/web/packages/dismo/index.html.

[4] Barton, K. 2012 MuMIn: Multi-model inference. Available: http://mumin.r-forge.r-project.org.

[5] Revell, L.J. 2012 phytools: An R package for phylogenetic comparative biology (and other things). . *Methods in Ecology and Evolution* **3**, 217-223.

Species	Parasite	virulence	status	rejection	cuckoo mass (g)	nest type (0=open)	Clutch size	host mass (g)	cuckoo/host mass	Latitude	Continent	References rejection rate	Reference clutch
Acanthiza chrysorrhoa	Chalcites lucidus	2	1	10	23	1	3.5	10	2.3	-35.1293	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Acanthiza pusilla	Cacomantis flabelliform	2	1	12.5	44	1	2.4	6	7.3333	-36.0056	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Acanthiza apicalis	Chalcites basalis	2	1	0	22	1	3	7	3.1429	-35.0056	Australia	Brooker & Brooker (1989)	Jetz et al. 2008
Acanthiza reguloides	Chalcites lucidus	2	1	0	23	1	3.9	7.5	3.0667	-34.548	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Acanthorhynchus tenuirostris	Cuculus pallidus	2	1	33.3	82	0	2	11.2	7.3214	-35.1379	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Acridotheres tristis	NA	0	NA	0	NA	0	9	115.6	NA	-7.47844	Asia	Begum *et al.* (2012)	Jetz et al. 2008
Acrocephalus arundinaceus	Cuculus canorus	2	1	42.4	115	0	4.2	21.9	5.2511	57.5393	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Acrocephalus palustris	Cuculus canorus	2	1	90	115	0	3.9	11.9	9.6639	57.34732	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Acrocephalus schoenobaenu	Cuculus canorus	2	1	20	115	0	5.5	10.8	10.648	59.18157	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Acrocephalus scirpaceus	Cuculus canorus	2	1	43.6	115	0	3.9	13	8.8462	55.45531	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Acrocephalus stentoreus	NA	0	NA	0	NA	0	4.2	NA	NA	23.50871	Australia	Brown *et al.* (1990)	Jetz et al. 2008
Agelaioides badius	Molothrus bonariensis	1	1	0	55.7	0	4	43.7	1.2746	-28.4169	America	Fraga (1998)	HBW
Agelaius phoeniceus	Molothrus ater	1	1	3.3	57.3	0	3	65.5	0.8748	36.48337	America	Rothstein (1975)	Jetz et al. 2008
Agelaius xanthomus	Molothrus bonariensis	1	1	0	55.7	0	3	41	1.3585	17.99046	America	Post & Wiley 1977	Jetz et al. 2008
Alauda arvensis	Cuculus canorus	2	0	10	115	0	7	35.1	3.2764	55.45454	Europe	Martín-Vivaldi *et al.* 2012	Jetz et al. 2008
Ammodramus humeralis	Molothrus bonariensis	0	NA	0	55.7	0	3.5	16	NA	-14.7956	America	Mason (1986)	Jetz et al. 2008
Ammodramus savannarum	Molothrus ater	1	1	0	57.3	1	4.5	17	3.3706	29.80852	America	Peer & Sealy (2004a)	Jetz et al. 2008
Anthochaera carunculata	Cuculus pallidus	2	1	42.9	82	0	2	108.5	0.7558	-36.3982	Australia	Langmore *et al.* (2005)	Jetz et al. 2008

Coccyzus erythropthalmus	Molothrus ater	0	NA	41.7	NA	0	3.5	NA	NA	37.55126	America	Rothstein (1975)	HBW
Contopus sordidulus	Molothrus ater	1	0	20	57.3	0	2.8	19	3.0158	31.01432	America	Peer & Sealy (2004a)	Jetz et al. 2008
Copsychus saularis	NA	0	NA	0	NA	0	3.2	33.2	NA	16.25333	Asia	Begum *et al.* (2012)	Jetz et al. 2008
Corvus corax	Clamator glandarius	0	NA	0	NA	0	4.6	1051.9	NA	59.10408	Europe	Soler (1990)	Jetz et al. 2008
Corvus corone	Clamator glandarius	1	1	0	124	0	4.5	536.5	0.2311	59.00317	Europe	M. Soler *et al.* (2001)	Jetz et al. 2008
Corvus monedula	Clamator glandarius	1	0	0	124	1	4	246	0.5041	58.84489	Europe	Soler (1990)	Jetz et al. 2008
Corvus splendens	Eudynamis scolopaceu	2	1	9.1	234	0	4.5	371	0.6307	16.94979	Asia	Begum *et al.* (2012)	Jetz et al. 2008
Cossypha caffra	NA	0	NA	0	NA	0	2.4	28.5	NA	-25.5287	Africa	Honza *et al.* (2005)	Jetz et al. 2008
Cyanopica cyanus	Cuculus canorus	2	1	34.7	115	1	5.9	94	1.2234	35.6338	Asia	Nakamura *et al.* (1998)	Jetz et al. 2008
Delichon urbicum		0	NA	0	NA	1	4.7	NA	NA	38.90253	Europe	Yang *et al.* (2014)	
Dendroica discolor	Molothrus ater	1	1	0	57.3	0	4	10.8	5.3056	33.71589	America	Rothstein (1975)	Jetz et al. 2008
Dendroica petechia	Molothrus ater	1	1	100	57.3	0	4.5	9.8	5.8469	32.41884	America	Briskie *et al.* (1992)	Jetz et al. 2008
Dicrurus adsimilis	Cuculus gularis	2	1	100	110	0	3	41	2.6829	-11.2988	Asia	Begum *et al.* (2012)	HBW
Dicrurus macrocercus	Cuculus micropterus	2	1	100	115	0	3.2	26.9	4.2751	19.20582	Africa	Noble (1995)	Jetz et al. 2008
Dumetella carolinensis	Molothrus ater	1	1	100	57.3	0	3.5	33	1.7364	30.71693	America	Rothstein (2001)	Jetz et al. 2008
Emberiza cia	Cuculus canorus	0	NA	100	NA	0	3.9	NA	NA	40.58222	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Emberiza cirlus	Cuculus canorus	0	NA	100	NA	0	3.2	25	NA	42.14608	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Emberiza citrinella	Cuculus canorus	2	0	95.2	115	0	3.9	30	3.8333	58.89037	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Emberiza schoeniclus	Cuculus canorus	2	0	95	115	0	4.5	19	6.0526	57.12787	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Empidonax minimus	Molothrus ater	1	1	0	57.3	0	4	10.5	5.4571	21.8754	America	Peer & Sealy (2004a)	Jetz et al. 2008

Eopsaltria australis	Molothrus ater	1	0	60	57.3	0	2.4	19.6	2.9235	-32.9274	Australia	Langmore *et al.*(2005)	Jetz et al. 2008
Erithacus rubecula	Cuculus canorus	2	1	16.1	115	1	4.9	18.2	6.3187	57.97443	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Erythropygia galactotes	Cuculus canorus	2	1	23.5	115	0	3.5	25.5	4.5098	23.83852	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Euphagus carolinus	Molothrus ater	1	0	7	57.3	0	4.5	55.7	1.0287	37.47758	America	Peer & Sealy (2004a)	Jetz et al. 2008
Euphagus cyanocephalus	Molothrus ater	1	1	18	57.3	0	5	68.5	0.8365	37.20917	America	Peer & Sealy (2004a)	Jetz et al. 2008
Euplectes orix	Chrysococcyx caprius	2	1	52	35	1	3	25	1.4	-22.106	Africa	Noble (1995)	HBW
Ficedula hypoleuca	NA	-1	NA	0	NA	1	5.7	14.5	NA	58.43228	Europe	Davies 2000	Jetz et al. 2008
Fringilla coelebs	Cuculus canorus	2	0	78.8	115	0	4.5	21.4	5.3738	58.30004	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Fringilla montifringilla	Cuculus canorus	2	1	88.1	115	0	5.9	24	4.7917	58.07395	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Furnarius rufus	Molothrus bonariensis	1	1	86.7	55.7	1	3.2	56.5	0.9858	-26.6318	America	Mason (1986)	Jetz et al. 2008
Gerygone fusca	Cacomantis flabelliform	2	0	25.5	44	1	2.5	6	7.3333	-32.0506	Australia	Langmore *et al.* (2005)	HBW
Gerygone olivacea	Chalcites minutillus	0	NA	0	NA	1	2.4	NA	NA	-29.2673	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Hippolais icterina	Cuculus canorus	2	0	66.7	115	0	4.5	14.6	7.8767	59.47097	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Hippolais pallida	Cuculus canorus	0	NA	87	NA	0	3	NA	NA	35.64012	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Hippolais polyglotta	Cuculus canorus	2	1	100	115	0	3.9	11	10.455	41.84307	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Hirundo rustica	Cuculus canorus	-1	NA	0	NA	0	5	18.6	NA	48.92227	Europe	Møller (1987)	Jetz et al. 2008
Hylocichla mustelina	Molothrus ater	1	1	0	57.3	0	3.5	47.4	1.2089	22.78234	America	Rothstein (1975)	HBW
Icteria virens	Molothrus ater	1	0	9	57.3	0	3.9	24.9	2.3012	26.78141	America	Peer & Sealy (2004a)	Jetz et al. 2008
Icterus bullockii	Molothrus ater	0	NA	100	NA	1	4.5	NA	NA	31.18688	America	Peer & Sealy (2004a)	Jetz et al. 2008
Icterus galbula	Molothrus ater	1	0	100	57.3	1	4.5	33.3	1.7207	24.71336	America	Rothstein (1977)	Jetz et al. 2008

Motacilla flava	Cuculus canorus	2	1	80	115	0	5	14.4	7.9861	54.60575	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Muscicapa striata	Cuculus canorus	2	0	66.7	115	0	3.5	15.4	7.4675	61.03328	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Neochmia temporalis	NA	-1	NA	0	NA	1	5	10.9	NA	-32.9643	Australia	Langmore *et al.* (2005)	HBW
Oenanthe oenanthe	Cuculus canorus	-1	NA	5.9	NA	0	5.3	23.5	NA	59.54277	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Oreoscoptes montanus	Molothrus ater	1	0	100	57.3	0	4.5	45	1.2733	37.20373	America	Rich & Rothstein (1985)	Jetz et al. 2008
Oriolus xanthornus	NA	0	NA	100	NA	1	2.8	NA	NA	16.27945	Asia	Begum *et al.* (2012)	Jetz et al. 2008
Pachycephala rufiventris	Cuculus pallidus	2	1	100	82	0	2.4	21.4	3.8318	-30.9944	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Paradoxornis alphonsianus	Cuculus canorus	2	1	80	115	0	4	10.5	10.952	37.76286		Yang (2010)	HBW
Paradoxornis webbianus	Cuculus canorus	2	1	100	115	0	5	9.75	11.795	30.53374	Asia	Lee & Yoo (2004).	HBW
Parus caeruleus	NA	-1	NA	0	NA	0	5.1	NA	NA	61.8842	Europe	Moksnes et al. 1991	Jetz et al. 2008
Parus major	NA	-1	NA	0	NA	1	7.7	16	NA	59.27457	Europe	Moksnes et al. 1991	Jetz et al. 2008
Parus palustris	NA	-1	NA	0	NA	0	7.1	NA	NA	50.91607	Europe	Moksnes et al. 1991	Jetz et al. 2008
Passer domesticus	NA	-1	NA	44.2	NA	1	8	23.26	NA	58.94109	Europe	M. Soler *et al.* (2011)	Jetz et al. 2008
Petroica goodenovii	Chalcites basalis	2	1	0	22	0	2.4	8.7	2.5287	-32.5726	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Phacellodomus sibilatrix	Molothrus bonariensis	1	0	0	55.7	0	3	15	3.7133	-27.9653	America	Mason (1986)	HBW
Phacellodomus striaticollis	Molothrus bonariensis	0	NA	0	55.7	1	3	24	NA	-33.7329	America	Mason (1986)	HBW
Pheucticus ludovicianus	Molothrus ater	1	1	0	57.3	0	4	45.6	1.2566	19.88788	America	Rothstein (1975)	HBW
Phleocryptes melanops	Molothrus bonariensis	1	0	0	55.7	1	2.8	14.2	3.9225	-26.5401	America	Mason (1986)	Jetz et al. 2008
Phoenicurus ochruros	Cuculus canorus	0	NA	100	NA	0	4.9	NA	NA	53.60294	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Phoenicurus phoenicurus	Cuculus canorus	2	0	37.9	115	1	5.9	12.1	9.5041	56.69015	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008

Phylidonyris novaehollandiae	Cuculus canorus	2	0	0	115	0	2.4	19.4	5.9278	-36.568	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Phylloscopus bonelli	Cuculus canorus	0	NA	50	NA	0	3.9	7.4	NA	43.23455	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Phylloscopus collybita	Cuculus canorus	2	0	100	115	1	5.5	7	16.429	51.41616	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Phylloscopus humei	Cuculus canorus	2	1	100	115	1	4.5	6.9	16.667	41.84621	Asia	Marchetti (2000)	Jetz et al. 2008
Phylloscopus trochilus	Cuculus canorus	2	0	90	115	1	5.7	9	12.778	58.00528	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Pica hudsonia	Molothrus ater	0	NA	100	NA	1	6.5	NA	NA	44.95102	America	Underwood *et al.* (2004)	HBW
Pica nuttalli	Molothrus ater	0	NA	100	NA	1	6.5	NA	NA	37.83052	America	Bolen *et al.*(2000)	HBW
Pica pica	Clamator glandarius	1	1	76.5	124	1	5.3	188.1	0.6592	59.11279	Europe	J.J. Soler *et al.* (1999a)	Jetz et al. 2008
Pitangus sulphuratus	Molothrus bonariensis	0	NA	25	55.7	1	3.9	71.5	NA	17.40953	America	Mason (1986)	Jetz et al. 2008
Ploceus cucullatus	Molothrus bonariensis	1	0	58.4	32	1	2.8	40.9	0.7824	-3.50522	America	Cruz *et al.* (2008)	Jetz et al. 2008
Ploceus philippinus	NA	0	NA	100	NA	1	3.2	NA	NA	15.97455	Asia	Dhindsa & Sandhu (1988)	Jetz et al. 2008
Ploceus taeniopterus	Molothrus bonariensis	0	NA	28	57.3	1	2.5	NA	NA	2.756095	Africa	Jackson 1998	HBW
Polioptila dumicola	Molothrus bonariensis	0	NA	0	55.7	0	3.9	6.5	NA	-27.667	America	Mason (1986)	Jetz et al. 2008
Pooecetes gramineus	Molothrus ater	1	0	0	57.3	0	3.9	25.7	2.2296	32.3474	America	Rothstein (1975)	Jetz et al. 2008
Progne tapera	Molothrus bonariensis	0	NA	0	55.7	1	4	35	NA	-15.9296	America	Mason (1986)	HBW
Protonotaria citrea	Molothrus ater	0	NA	0	NA	0	4.9	15.5	NA	24.75996	America	Peer & Sealy (2004a)	Jetz et al. 2008
Prunella modularis	Cuculus canorus	2	1	3.1	115	0	4.9	19.7	5.8376	56.62999	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Psarocolius montezuma	Molothrus oryzivorus	1	1	72	174	0	2	376.5	0.4622	12.37827	America	Cunningham & Lewis (2005)	HBW
Pycnonotus capensis	Clamator jacobinus	1	1	0	66	0	2.4	47.5	1.3895	-33.6357	Africa	Krüger (2011)	Jetz et al. 2008
Pyrocephalus rubinus	Molothrus bonariensis	0	NA	0	55.7	0	2.5	12.7	NA	24.71574	America	Mason (1986)	HBW

Pyrrhocorax pyrrhocorax	Clamator glandarius	1	0	0	124	1	3.9	324	0.3827	39.47844	Europe	Soler (1990)	Jetz et al. 2008
Pyrrhula pyrrhula	NA	-1	NA	0	NA	0	4.5	23	NA	59.25502	Europe	Davies 2000	Jetz et al. 2008
Quiscalus major	Molothrus ater	0	NA	100	NA	0	2.7	158.8	NA	29.53546	America	Peer & Sealy (2004*a*)	HBW
Quiscalus mexicanus	Molothrus ater	0	NA	100	NA	0	3.5	168.7	NA	27.77338	America	Peer & Rothstein (2010)	Jetz et al. 2008
Quiscalus quiscula	Molothrus ater	0	NA	11.9	NA	0	4.2	82	NA	34.17065	America	Peer & Rothstein (2010)	Jetz et al. 2008
Rhipidura fuliginosa	Cacomantis variolosus	2	1	100	34	0	3.4	8	4.25	-41.0075	Australia	Langmore *et al.* (2005)	HBW
Rhipidura leucophrys	Cuculus pallidus	2	1	100	82	0	2.8	27.7	2.9603	-31.2645	Australia	Langmore *et al.* (2005)	Jetz et al. 2008
Satrapa icterophrys	Molothrus bonariensis	0	NA	0	55.7	0	2.8	20	NA	-25.6268	America	Mason (1986)	Jetz et al. 2008
Saxicola torquatus	Cuculus canorus	2	0	0	115	0	4.9	15.3	7.5163	16.88782	Europe	Martín-Vivaldi *et al.* (2012)	Jetz et al. 2008
Sayornis phoebe	Molothrus ater	1	1	6	57.3	0	4.5	18.1	3.1657	31.98566	America	Rothstein (1975)	Jetz et al. 2008
Serpophaga subcristata	Molothrus bonariensis	0	NA	0	55.7	0	2	6.5	NA	-29.9376	America	Mason (1986)	HBW
Sialia currucoides	Molothrus ater	1	0	20	57.3	1	5.5	30	1.91	36.90721	America	Peer & Sealy (2004a)	Jetz et al. 2008
Spiza americana	Molothrus ater	1	1	11	57.3	0	3.5	27	2.1222	29.84484	America	Peer & Sealy (2004a)	Jetz et al. 2008
Spizella pallida	Molothrus ater	1	1	0	57.3	0	3.9	15	3.82	32.14297	America	Peer & Sealy (2004a)	Jetz et al. 2008
Spizella passerina	Molothrus ater	1	1	15.8	57.3	0	4	15.5	3.6968	32.83681	America	Rothstein (1975)	Jetz et al. 2008
Spizella pusilla	Molothrus ater	1	1	0	57.3	0	4.2	15.7	3.6497	35.73388	America	Peer & Sealy (2004a)	Jetz et al. 2008
Sturnella magna	Molothrus ater	1	1	33.3	57.3	1	5	135	0.4244	32.89269	America	Rothstein (1975)	Jetz et al. 2008
Sturnella neglecta	Molothrus ater	0	NA	100	NA	1	4.2	103.5	NA	37.22731	America	Peer & Sealy (2004a)	Jetz et al. 2008
Sturnus contra	NA	0	NA	0	NA	1	5	NA	NA	23.24311	Asia	Begum *et al.* (2012)	HBW
Sturnus vulgaris	NA	-1	NA	7	NA	1	6	58.35	NA	57.71829	Europe	Moksnes et al. 1991	Jetz et al. 2008

Chapter 4

Brood parasitism and the evolution of cooperative breeding in birds

The results from this chapter are now published in *Science (2013)*, and it was done in collaboration with several researchers. My contribution was the comparative analyses in Africa and Australia to test the association between cooperative breeding and being a host and Figure 2. Originally, the comparative analyses were planned as an independent publication, but we decided to combine these with other results and submit a stronger paper. After presenting the article I show the detailed methods I used for the comparative analyses.

Brood Parasitism and the Evolution of Cooperative Breeding in Birds

W. E. Feeney,[1] I. Medina,[1] M. Somveille,[2] R. Heinsohn,[3] M. L. Hall,[4] R. A. Mulder,[4] J. A. Stein,[3] R. M. Kilner,[2] N. E. Langmore[1]*

The global distribution of cooperatively breeding birds is highly uneven, with hotspots in Australasia and sub-Saharan Africa. The ecological drivers of this distribution remain enigmatic yet could yield insights into the evolution and persistence of cooperative breeding. We report that the global distributions of avian obligate brood parasites and cooperatively breeding passerines are tightly correlated and that the uneven phylogenetic distribution of cooperative breeding is associated with the uneven targeting of hosts by brood parasites. With a long-term field study, we show that brood parasites can acquire superior care for their young by targeting cooperative breeders. Conversely, host defenses against brood parasites are strengthened by helpers at the nest. Reciprocally selected interactions between brood parasites and cooperative breeders may therefore explain the close association between these two breeding systems.

Cooperative breeding, in which three or more individuals contribute to the care of young in the nest, occurs in around 9% of birds (*1*). The distribution of this social system is strongly skewed toward two major hotspots: Australasia and sub-Saharan Africa (*2*) (Fig. 1A). Ecological correlates of this distribution include both variable, unpredictable environmental conditions (*2*) and stable, predictable conditions (*3*). Unsurprisingly, the broad-scale ecological conditions that favor the evolution and persistence of cooperative breeding in birds therefore remain controversial (*2*, *4*, *5*).

Previous studies have proposed that cooperatively breeding species are more likely to be hosts of avian interspecific brood parasites than are noncooperative species (*6*, *7*). We investigated the correlation between avian brood parasitism and cooperative breeding. Interspecific brood parasites lay their eggs in the nests of other birds, primarily passerines, and abandon their young to the care of the host (*8*). The cost of hosting a brood parasite can be immense, so hosts are typically under selection to evolve defenses against parasitism (*8*). One of the most ubiquitous host defenses is the mobbing of brood parasites (*9*). Collective mobbing by multiple individuals can provide a more effective defense than solitary or pair mobbing, thus providing a selective force for cooperative or colonial breeding (*10*).

To test this hypothesis, we first compared the global geographic breeding distribution of avian brood parasites and cooperatively breeding passerine species (*11*). We found a strong correlation between species richness in cooperative breeders and species richness in brood parasites [simultaneous autoregressive model (z) = 61.3, $P < 0.0001$, correlation coefficient (r^2) = 0.68, Fig. 1], with both exhibiting the same geographic skew toward sub-Saharan Africa and Australasia [63% of avian brood parasite species breed exclusively within this region (*8*)]. This correlation remains strong after controlling for avian species richness ($z = 21.0$, $P < 0.0001$, $r^2 = 0.41$, fig. S1).

This correlation could reflect a direct association between brood parasitism and cooperative breeding, or both breeding systems could be the outcome of a third variable, such as the high cost of parental care in variable environments (*2*). If there is a direct association, either because exploitation by brood parasites promotes cooperative breeding or because brood parasites favor cooperatively breeding hosts, we would predict that within a given geographic region, species that are hosts of brood parasites should be more likely to breed cooperatively than nonhosts. We tested this prediction using phylogenetic comparative methods for two regions with sufficiently well-studied avifaunas: Australia and southern Africa (*11*). These two regions encompass the phylogenetically diverse passerine and nonpasserine hosts of 21 cuckoo species, 6 honeyguide species, and 9 parasitic finch species. We used published classifications of the host status of Australian passerines [brood parasites exploit passerines exclusively in Australia (*12*)] and all southern African birds (*13*) and the modes of parental care in all bird species worldwide (*1*). Our analyses revealed a significant association between hosts of brood parasites and cooperative breeders in both southern Africa (Bayes factor = 18.36, strongly correlated; likelihood ratio test: $\chi^2 = 60.28$, $P < 0.001$; Fig. 2A) and Australia (Bayes factor = 17.34, strongly correlated; like-

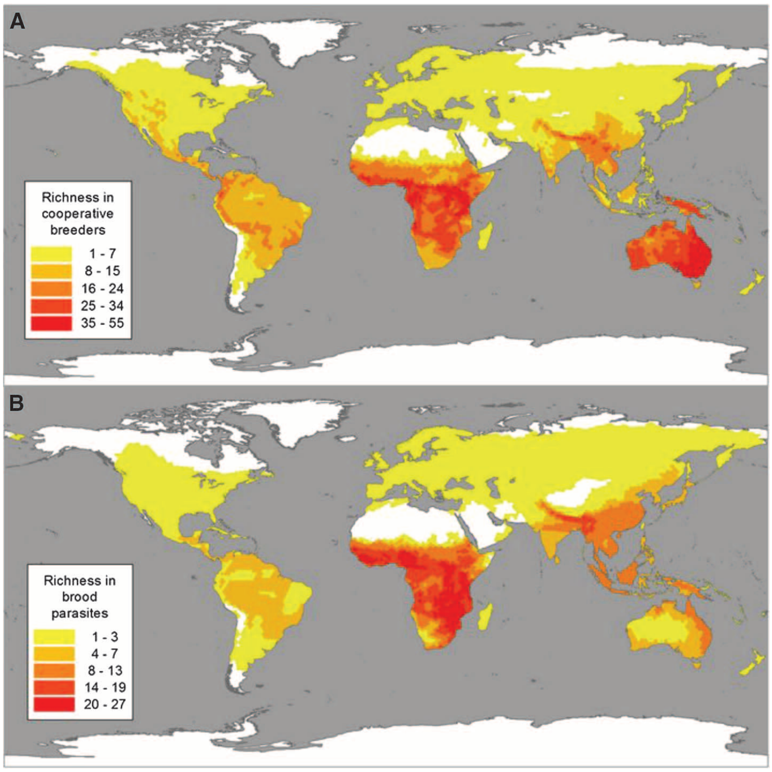


Fig. 1. Global patterns of richness in (A) avian cooperatively breeding passerine species and (B) obligate avian brood parasite species, during their breeding seasons.

---

[1]Research School of Biology, The Australian National University, Canberra, Australia. [2]Department of Zoology, University of Cambridge, Cambridge, UK. [3]The Fenner School of Environment and Society, The Australian National University, Canberra, Australia. [4]Department of Zoology, University of Melbourne, Melbourne, Australia.

*Corresponding author. E-mail: naomi.langmore@anu.edu.au

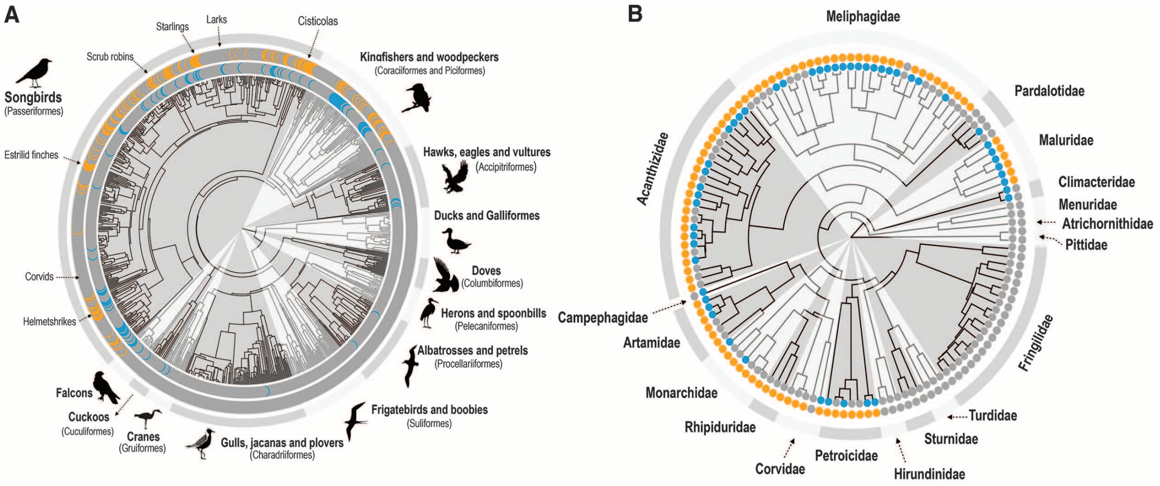


Fig. 2. Random phylogenetic trees for (A) 892 bird species in southern Africa (gray divisions represent orders) and (B) 129 passerine species in Australia (gray divisions represent families). Orange circles indicate cuckoo hosts, and blue circles indicate cooperative breeders.

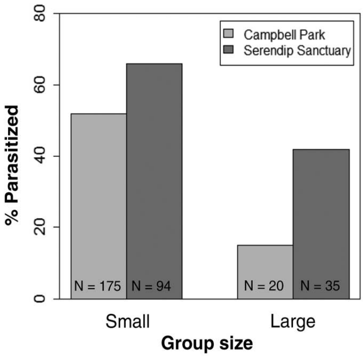


Fig. 3. The percentage of large and small superb fairy-wren groups that were parasitized by Horsfield's bronze cuckoos at Campbell Park and Serendip Sanctuary, Australia.

lihood ratio test: $\chi^2 = 11.66$, $P = 0.02$; Fig. 2B). In southern Africa, 27.5% of hosts were cooperative breeders, compared to only 7.6% of nonhosts. Similarly, in Australia 52.8% of hosts were cooperative breeders, compared to 11.9% of nonhosts.

Three non–mutually exclusive processes could explain why brood parasite hosts are more likely to be cooperative breeders: (i) Brood parasites might selectively target cooperative breeders to maximize the care of their offspring (*7*); (ii) cooperative breeders may be more obvious targets as a result of the increased activity of helpers near the nest (*6*, *7*); and (iii) cooperative breeders may be better able to defend their nests against brood parasitism (*7*), selecting for cooperative breeding in hosts. To investigate whether one or more of these processes underpin the patterns uncovered by our comparative analysis, we conducted field observations and experiments on the facultatively cooperative superb fairy-wren *Malurus cyaneus*. In this species, some pairs breed unassisted, whereas others are assisted by up to six nonbreeding helpers. This allowed us to investigate how cooperative breeding might change the outcome of interactions with brood parasites. In southeastern Australia, superb fairy-wrens are the primary host of Horsfield's bronze cuckoo, *Chalcites basalis* (*12*), and can suffer high annual rates of brood parasitism (*14*).

We began by investigating whether cuckoos might gain a selective advantage by preferentially targeting cooperative breeders for parasitism, using superb fairy-wren breeding and parasitism data (*11*). Cuckoo chicks grew slightly faster when reared by groups of three or more ($n = 30$ cuckoo chicks, day of the nestling period × group size; $F_1 = 7.46$, $P = 0.009$), with a predicted mean (±SE) mass on day 12 of 22.6 g (±0.5 g) if reared by a pair and 23.4 g (±0.5 g) if reared by a group. The chance of surviving to fledge was also greater for nestlings reared by larger groups, because predation rates decreased with increasing group size [generalized linear mixed model (GLMM): $\chi^2_1 = 4.31$, $P = 0.04$]. Although superb fairy-wrens commonly reject cuckoo chicks (*14*), the incidence of chick rejection was not correlated with group size ($n = 72$ cuckoo chicks, logistic regression, $\chi^2_1 = 0.6$, $P = 0.44$). Overall, then, our analyses provide support for hypothesis (i). We find that brood parasites can gain a fitness advantage for their offspring by associating with cooperative breeders, because they offer superior provisioning and a more effective defense against predators.

However, our analyses also show that this potential fitness advantage was seldom realized by Horsfield's bronze cuckoos parasitizing superb fairy-wrens, even when considering data from two sites over 500 km apart. Large groups were significantly less likely to be parasitized than small groups at both Campbell Park (GLMM: $\chi^2_1 = 7.68$, $P = 0.006$) and Serendip Sanctuary ($\chi^2_1 = 5.01$, $P = 0.027$; Fig. 3). Therefore, our results do not support hypothesis (ii): Cuckoos were not drawn to parasitize cooperative breeders because they are more salient targets for exploitation. Instead, we find support for hypothesis (iii), because cooperative breeding facilitates defense against brood parasites. We quantified the fitness advantage associated with better defenses against parasitism in large groups using data from Campbell Park (*11*). Relative to small groups, the reduced probability of parasitism in large groups increases the production of young by 0.2 fledglings per group per season. Therefore, both parents and related helpers gain a fitness advantage from cooperative breeding when interacting with brood parasites.

Subsequent experimental analyses of behavior at the nest revealed how larger groups are able to escape parasitism more frequently than smaller groups. We found that superb fairy-wrens were more aggressive toward mounts of a cuckoo than of a nest predator (eastern brown snake, *Pseudonaja textilis*), a predator of adult birds (collared and Eurasian sparrowhawk, *Accipiter cirrocephalus* and *A. nisus*, respectively), a predator of both adults and nestlings (pied currawong, *Strepera graculina*), or a nonthreatening control (white-plumed honeyeater, *Lichenostomus penicillatus*; linear mixed effects model on number of mobbing calls: $\chi^2_4 = 53.95$, $P < 0.0001$; Fig. 4A). Further, cuckoo-targeted mobbing was elicited by a referential vocalization. Superb fairy-wrens produce whining alarm calls (*15*) that are structurally unlike any other calls in their repertoire (Wilk's $\lambda = 0.11$, exact $F_{8,56} = 13.75$, $P < 0.01$; fig. S3) and do so exclusively when confronting a cuckoo (Friedman test: $\chi^2_4 = 54.72$, $P < 0.0001$; Fig. 4C). With playback experiments, we found that this call elicits a more rapid approach by group members than mobbing alarm calls or a

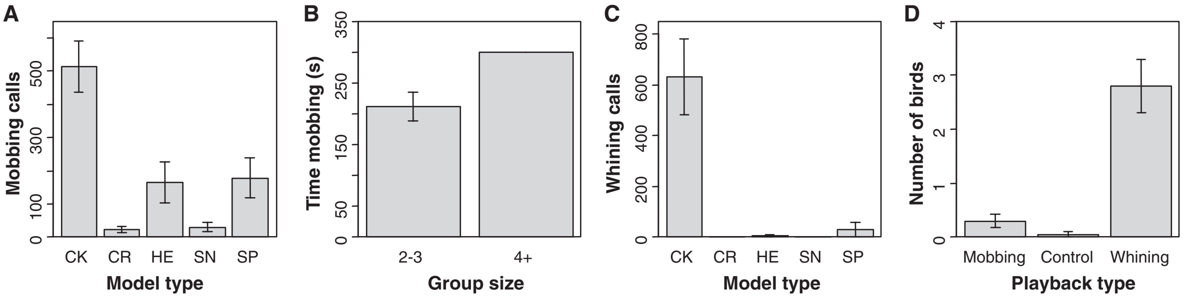


Fig. 4. (A) Mean number of mobbing alarm calls produced by 15 fairy-wren groups in response to different model types: cuckoo (CK), currawong (CR), honeyeater (HE), snake (SN), and sparrowhawk (SP). (**B**) Mean time spent mobbing the cuckoo model (<0.5 m from the model) by small (*n* = 27 groups) versus large (*n* = 5 groups) groups. (**C**) Mean number of whining calls produced in response to the five model types. (**D**) Mean number of individuals that approached playbacks (*n* = 20 each) of fairy-wren mobbing alarm calls, a control sound, and fairy-wren whining alarm calls. Error bars denote the standard error.

control sound (a parrot call, GLMM: $\chi^2_2 = 68.05$, $P < 0.0001$; Fig. 4D). Once mobilized, the strength of these defenses increases with group size. Large groups were more vigilant around their nest (GLMM: $\chi^2_1 = 8.03$, $P < 0.004$), spent more time mobbing the cuckoo than smaller groups (Kruskal Wallis test: $\chi^2_1 = 5.42$, $P = 0.02$; Fig. 4B), and ultimately were less likely to be parasitized. Thus, superb fairy-wrens possess cuckoo-specific nest defenses, which are enhanced by helper contributions and which can explain the lower parasitism rates experienced by large groups.

Our findings show a pronounced association between avian brood parasitism and cooperative breeding in birds, on a global scale. Our field data suggest that a two-way process underpins this relationship. On the one hand, brood parasites can gain a fitness advantage by preferentially exploiting the superior care provided by cooperatively breeding groups. On the other hand, the genetic relatives of offspring raised by cooperatively breeding families potentially gain fitness from the superior defenses that the extended family collectively mounts against brood parasites. Defense against brood parasitism is therefore an important kin-selected fitness advantage associated with cooperative breeding [see also (*16*)]. In superb fairy-wrens, we have shown this two-way process at work, but in other cooperatively breeding hosts, especially those with a less protracted coevolutionary relationship with brood parasites, only the first part of the process may be evident. The challenge remaining for future work is to determine the extent to which brood parasites have influenced the biology of cooperatively breeding species.

References and Notes

1. A. Cockburn, *Proc. Biol. Sci.* **273**, 1375–1383 (2006).
2. W. Jetz, D. R. Rubenstein, *Curr. Biol.* **21**, 72–78 (2011).
3. S. T. Emlen, *Am. Nat.* **119**, 29 (1982a).
4. A. Cockburn, A. F. Russell, *Curr. Biol.* **21**, R195–R197 (2011).
5. J.-C. T. Gonzalez, B. C. Sheldon, J. A. Tobias, *Proc. Biol. Sci.* **280**, 20131297 (2013).
6. A. Monadjem, *S. Afr. J. Sci.* **92**, 295 (1996).
7. A. Poiani, M. A. Elgar, *Anim. Behav.* **47**, 697–706 (1994).
8. N. B. Davies, *Cuckoos, Cowbirds and Other Cheats* (T & A D Poyser, London, 2000).
9. W. E. Feeney, J. A. Welbergen, N. E. Langmore, *Anim. Behav.* **84**, 3–12 (2012).
10. M. Brown, M. J. Lawes, *Ibis* **149**, 321–327 (2007).
11. Materials and methods are available as supplementary materials on *Science* online.
12. M. G. Brooker, L. C. Brooker, *Aust. Zool. Rev.* **2**, 1 (1989).
13. P. A. R. Hockey, W. R. J. Dean, P. G. Ryan, *Roberts Birds of Southern Africa* (The Trustees of the John Voelcker Bird Book Fund, Cape Town, South Africa, ed. 7, 2005).
14. N. E. Langmore, S. Hunt, R. M. Kilner, *Nature* **422**, 157–160 (2003).
15. N. E. Langmore *et al.*, *Behav. Ecol.* **23**, 798–805 (2012).
16. D. Canestrari, J. M. Marcos, V. Baglione, *Anim. Behav.* **77**, 1337–1344 (2009).

Acknowledgments: We thank our field assistants, L. Joseph for assistance with specimens, and A. Cockburn for helpful discussions. W.E.F. was supported by the Canberra Birds Conservation Fund and Australian Geographic. R.A.M. and M.L.H. were supported by Australian Research Council (ARC) Discovery Grant DP110103120. N.E.L. was supported by ARC Discovery Grant DP110101966. Data are archived on Figshare. N.E.L. and W.E.F. conceived the study; macroecological analyses were done by M.S. and J.A.S.; comparative analyses were done by I.M.; field data were collected by N.E.L., R.M.K., R.H., M.L.H., and R.A.M.; model and playback experiments were done by W.E.F.; and N.E.L. and R.M.K. wrote the paper.

Supplementary Materials
www.sciencemag.org/content/342/6165/1506/suppl/DC1
Materials and Methods
Figs. S1 to S3
Table S1
References (*17–24*)

3 May 2013; accepted 6 November 2013
10.1126/science.1240039

C57BL/6N Mutation in *Cytoplasmic FMRP interacting protein 2* Regulates Cocaine Response

Vivek Kumar,[1,2] Kyungin Kim,[1] Chryshanthi Joseph,[1] Saïd Kourrich,[3]* Seung-Hee Yoo,[1]* Hung Chung Huang,[1] Martha H. Vitaterna,[4] Fernando Pardo-Manuel de Villena,[5] Gary Churchill,[6] Antonello Bonci,[3,7] Joseph S. Takahashi[1,2]‡

The inbred mouse C57BL/6J is the reference strain for genome sequence and for most behavioral and physiological phenotypes. However, the International Knockout Mouse Consortium uses an embryonic stem cell line derived from a related C57BL/6N substrain. We found that C57BL/6N has a lower acute and sensitized response to cocaine and methamphetamine. We mapped a single causative locus and identified a nonsynonymous mutation of serine to phenylalanine (S968F) in *Cytoplasmic FMRP interacting protein 2* (*Cyfip2*) as the causative variant. The S968F mutation destabilizes CYFIP2, and deletion of the C57BL/6N mutant allele leads to acute and sensitized cocaine-response phenotypes. We propose that CYFIP2 is a key regulator of cocaine response in mammals and present a framework to use mouse substrains to identify previously unknown genes and alleles regulating behavior.

The reference mouse strain, C57BL/6J, was established in 1921 and has been maintained at the Jackson Laboratory since 1948 (*1*). In 1951, a colony of C57BL/6J was shipped to the National Institutes of Health (NIH), and C57BL/6N became a second major source of C57BL/6 mice. Large-scale projects use different C57BL/6 substrains, including the International Knockout Mouse Consortium (IKMC), which uses C57BL/6N embryonic stem (ES) cells (*2*, *3*), and

SUPPLEMENTARY MATERIAL

Comparative analyses

We conducted our phylogenetic comparative analyses on the Australian and southern African avifauna because there are well-studied and comprehensive classifications of host status are published for these regions (12, *13).* For our analysis of Australian hosts, we used Brooker and Brooker's published compilation of 5,244 records of parasitism of 204 passerines in Australia (brood parasites in Australia exploit passerines exclusively, *12).* To overcome biases in any one source of data, Brooker and Brooker combined records of parasitism from the literature, museum and private egg collections, the Royal Australian Ornithologist's Union nest records scheme, the Australian Bird Banding Scheme and unpublished records of parasitism. Moreover, they applied rigorous criteria to their classification of hosts as biological (successful) hosts as distinct from accidental or unsuitable hosts; biological hosts had multiple (>4), independent (>1 observer, > 1 location, > 1 year) records of parasitism, and either 1) an egg or authentic nestling record followed through to fledgling; 2) nestling and feeding records as well as egg records; or 3) egg records alone, if congeneric with a known biological host of the cuckoo. For our analysis, we included all passerine species classified as either non-hosts or biological hosts of Australian cuckoos (12). We excluded species classified as 'non- biological' (occasional or rare) hosts, because it was not possible to infer the extent of selection on these hosts by brood parasites. Thus our dataset comprised 129 passerine species that were classified as either cuckoo hosts or non-hosts (87 and 42 species respectively). For our analysis of southern African hosts, we used the host listings for all brood parasites in 'Roberts Birds of Southern Africa' (13). These lists mostly do not distinguish between regular and occasional hosts, so we included all species listed as hosts and excluded those listed as provisional hosts. The honeyguides (Family Indicatoridae) in southern Africa exploit non-passerine hosts, so this analysis included both passerine and non-passerine species. Our southern African dataset comprised 138 host species and 754 non-host species.

Information on breeding system was obtained from Cockburn (1). This study inferred breeding systems for those species where it was unknown, based on the breeding systems of their closest relatives. To test the association between host status and social system, we downloaded 1000 avian phylogenetic trees from the 10,000 trees available at www.birdtree.org (20) and employed the Discrete module in the BayesTraits program (21). We used the maximum likelihood algorithm to estimate trait changes on our evolutionary tree and compared the likelihood of a model that assumed independent evolution of the two characters (host status and social system) with that of a model of correlated evolution between these characters. Likelihoods were calculated for each of the 1000 trees, and were then averaged for each model. Average likelihoods were compared using a likelihood ratio test (2* difference in log-likelihood) and the significance of the difference between both models was calculated following a chi-square distribution for four degrees of freedom (equal to the difference of parameters used by each model).

To confirm our results we also used the Bayesian inference algorithm, with 5,050,000 iterations for the Australian dataset (burn in 50,000) and 100,000,000 iterations for the African dataset (burn in 10,000,000). The sample interval was set at 1000 to achieve a low autocorrelation. We obtained a Bayesian factor by subtracting the likelihoods for each model and we used the table provided in the manual to assess the significance of the results.

Although phylogenetic methods allow exploration of the sequence of evolution of correlated traits, these methods rely strongly on ancestral character reconstruction, which has proved to be a problematic topic (22). Further, host status constitutes a particularlydifficult trait to reconstruct, given its lability and its tendency to be the product of convergence. Brood parasites tend to exploit species that are closely-related (particularly members of the same genus). This could lead to a false result that brood parasitism occurred early in the evolution of a clade of hosts, when in fact it might reflect recent annexation by a brood parasite of several closely-related species as hosts. Therefore this analysis was not appropriate here.

References

12. M. G. Brooker, L. C. Brooker, Cuckoo hosts in Australia. *Aust. Zool. Rev.* **2,** 1 (1989).

13. P. A. R. Hockey, W. R. J. Dean, P. G. Ryan, *Roberts Birds of Southern Africa* (The Trustees of the John Voelcker Bird Book Fund, Cape Town, South Africa, ed. 7, 2005).

14. N. E. Langmore, S. Hunt, R. M. Kilner, Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422,** 157–160 (2003). doi:10.1038/nature01460 Medline

15. N. E. Langmore, W. E. Feeney, J. Crowe-Riddell, H. Luan, K. M. Louwrens, A. Cockburn, Learned recognition of brood parasitic cuckoos in the superb fairy-wren *Malurus cyaneus*. *Behav. Ecol.* **23,** 798–805 (2012). doi:10.1093/beheco/ars033

16. D. Canestrari, J. M. Marcos, V. Baglione, Cooperative breeding in carrion crows reduces the rate of brood parasitism by great spotted cuckoos. *Anim. Behav.* **77,** 1337–1344 (2009). doi:10.1016/j.anbehav.2009.02.009

17. M. Somveille, A. Manica, S. H. M. Butchart, A. S. L. Rodrigues, Mapping global diversity patterns for migratory birds. *PLoS ONE* **8,** e70907 (2013). doi:10.1371/journal.pone.0070907 Medline

18. K. Sahr, D. White, A. J. Kimerling, Geodesic discrete global grid systems. *Cartogr. Geogr. Inform.* **30,** 121–134 (2003). doi:10.1559/152304003100011090

19. W. D. Kissling, G. Carl, Spatial autocorrelation and the selection of simultaneous autoregressive models. *Glob. Ecol. Biogeogr.* **17,** 59 (2008).

20. W. Jetz, G. H. Thomas, J. B. Joy, K. Hartmann, A. O. Mooers, The global diversity of birds in space and time. *Nature* **491,** 444–448 (2012). doi:10.1038/nature11631 Medline

21. M. Pagel, A. Meade, Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* **167,** 808–825 (2006). doi:10.1086/503444 Medline

22. T. R. Schultz, G. A. Churchill, The role of subjectivity in reconstructing ancestral character states: A Bayesian approach to unknown rates, states and transformation asymmetries. *Syst. Biol.* **48,** 651–664 (1999). doi:10.1080/106351599260229

Chapter 5

Batten down the thatches: Front-line defences in an apparently defenceless cuckoo host

This chapter constitutes the first part of my fieldwork in Canberra, Australia. The following is the article published in *Animal Behaviour*.

Animal Behaviour 112 (2016) 195–201

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Batten down the thatches: front-line defences in an apparently defenceless cuckoo host

Iliana Medina*, Naomi E. Langmore

Department of Ecology, Evolution and Behaviour, Australian National University, Canberra, Australia

ARTICLE INFO

Article history:
Received 10 September 2015
Initial acceptance 6 October 2015
Final acceptance 30 October 2015
Available online
MS. number: 15-00783R

Keywords:
breeding phenology
brood parasitism
cuckoos
front-line defences
mobbing

Avian brood parasites lay their eggs in the nests of other species, imposing high costs on their hosts. In theory, this should select for the evolution of defences against parasitism in hosts, yet eviction of parasite eggs or chicks is absent in many host species. One such host is the yellow-rumped thornbill, *Acanthiza chrysorrhoa*, the primary host of the shining bronze-cuckoo, *Chalcites lucidus*, in Australia. Here we tested whether the lack of egg and chick rejection in yellow-rumped thornbills has led to the evolution of alternative defences against brood parasitism. We provide evidence that this host has evolved two types of frontline defences. First, yellow-rumped thornbills responded to the presence of a cuckoo mount near the nest with mobbing behaviour and by occupying their dome-shaped nests, potentially as a means of blocking the small entrance hole. Second, we show that brood parasitism imposes directional selection for early breeding on the yellow-rumped thornbill and that yellow-rumped thornbills show a concomitant shift in their breeding phenology, breeding earlier than both congeneric and sympatric species. Our results highlight the importance of studying apparently defenceless hosts in order to identify alternative defence strategies.

Brood parasites lay their eggs in nests of other species, thus avoiding the costs of raising their own offspring (Davies, 2000). Cuckoos are particularly virulent brood parasites (Kilner, 2005), and when their chick hatches it usually evicts all the other eggs of its foster parents, resulting in extremely high costs to hosts. This strongly antagonistic interaction between hosts and cuckoos has led to the evolution of host strategies to prevent cuckoo parasitism, which have, in turn, selected for counter-adaptations in cuckoos to circumvent host defences (Davies, 2011).

As a defence against parasitism, many hosts evict brood parasite eggs from the nest, or they might even abandon the nest and build a new one (Davies, 2000). Although rejection of a foreign egg allows the host's own breeding attempt to continue, the host still typically loses one egg because the cuckoo female usually removes one host egg when she lays her own (Davies, 2000). Egg rejection is possibly the best-studied type of host defence strategy, and it has led to the evolution of egg mimicry by parasites (Aviles et al., 2012; Landstrom, Langmore, & Heinsohn, 2010; Spottiswoode, Stryjewski, Quader, Colebrook-Robjent, & Sorenson, 2011). Similarly, hosts may also reject the parasite chick (Langmore, Hunt, & Kilner, 2003; Sato, Tokue, Noske, Mikami, & Ueda, 2010; Tokue & Ueda, 2010), and this in turn has selected for the evolution of brood parasite chicks that mimic the appearance of host chicks (Langmore et al., 2011). Nevertheless, almost 40% of the main hosts of brood parasites are unable to recognize and reject parasitic eggs (Medina & Langmore, 2015) and cuckoo chick rejection is rare (Davies, 2011). Possible explanations for the lack of defences in some species are that the evolution of such defences may be too costly to evolve in some species, or there has been insufficient time for these defences to evolve (e.g. 'evolutionary lag hypothesis', Takasu, 1998). Another possibility is that the host has evolved effective defences, but at other stages of the breeding cycle.

Several 'frontline' defence strategies are used by hosts in response to parasitism. These are defences deployed before parasitism occurs, and they are potentially the most beneficial defences to hosts, because the host's entire clutch is preserved if frontline defences are successful. Host species may attempt to prevent parasitism physically, by mobbing the female brood parasite (Davies & Welbergen, 2008; Feeney, Welbergen, & Langmore, 2012; Gloag, Fiorini, Reboreda, & Kacelnik, 2013; Krüger, Davies, & Sorenson, 2007; Langmore et al., 2012; Tong, Horrocks, & Spottiswoode, 2015; Welbergen & Davies, 2011). Physical attacks can successfully prevent parasitism (Krüger, 2011; Neudorf & Sealy, 1994; Webster, 1994) and may even result in the death of the

---

* Correspondence: I. Medina, Department of Ecology, Evolution and Behaviour, Australian National University, Canberra 0200, Australia.
E-mail address: iliana.medina@anu.edu.au (I. Medina).

http://dx.doi.org/10.1016/j.anbehav.2015.12.006

parasite (Gloag et al., 2013; Molnár, 1944; Moyer, 1980). Although such behaviours may represent some energetic cost to the host, the overall outcome should be beneficial if cuckoo egg laying is prevented. Mobbing behaviour has been well studied in some cooperative breeders, such as superb fairy-wrens, *Malurus cyaneus*, where it has been found that large groups mob cuckoos more aggressively and experience lower rates of parasitism than small groups (Feeney et al., 2013). However, the degree of mobbing success for hosts that breed in pairs or small groups requires further investigation (but see Welbergen & Davies, 2009).

Another 'frontline' defence strategy may involve hosts avoiding brood parasitism by adjusting their breeding phenology to breed when cuckoos are not present (Boves, Sperry, Comolli, & Weatherhead, 2014; Brooker & Brooker, 1989a, 1989b). This idea has been poorly explored (Feeney et al., 2012) but has been suggested in some hosts, such as the red wattlebird, *Anthochaera carunculata*, which is exploited by the pallid cuckoo, *Cuculus pallidus* (Brooker & Brooker, 1989a, 1989b). Red wattlebirds breed at the same time as pallid cuckoos in southeastern Australia, where they are not a major host of the cuckoo, but commence breeding 3 months earlier than the cuckoo in southwestern Australia, where they are a primary host (Brooker & Brooker, 1989a, 1989b). A rationale for being an early breeder is that it may offer an alternative defence to hosts when the evolution of other types of defences is constrained.

Here, we tested for two types of frontline defences in the yellow-rumped thornbill, *Acanthiza chrysorrhoa* (Family: Acanthizidae), the primary host of the shining bronze-cuckoo, *Chalcites lucidus*, in Australia (Brooker & Brooker, 1989a, 1989b). Shining bronze-cuckoos are a highly virulent parasite: the cuckoo nestling evicts the host's eggs and then becomes the sole beneficiary of the host's care for over a month. Yellow-rumped thornbills build dome-shaped nests with very dark interiors (Langmore & Kilner 2009), which may constrain the evolution of egg rejection in this species (Langmore et al., 2005) and favour the evolution of alternative defences. Two previous studies showed that yellow-rumped thornbills are indeed poor egg rejecters, with rejection of nonmimetic model eggs recorded at 0% (Brooker & Brooker, 1989a, 1989b) and 10% (Langmore et al., 2005) of experimental nests. Moreover, there is no evidence of cuckoo chick rejection in this species ($N = 15$ shining bronze-cuckoo chicks reared to fledging, none rejected, N.E. Langmore, n.d.). Thus, contrary to theoretical expectations that cuckoo parasitism should select for host defences, previous studies suggest that yellow-rumped thornbills are defenceless cuckoo hosts (Langmore & Kilner, 2009; Langmore et al., 2005). However, there is no information on frontline defence strategies in this species. Yellow-rumped thornbills are well known as early breeders, and can be observed 'carrying nesting material through the freezing fog, just one day after the winter solstice' (McComas (1992), p. 140). Here we explored whether yellow-rumped thornbills have evolved frontline defences and whether this can explain the anomaly of an apparently defenceless, yet heavily exploited host. Specifically, we tested whether yellow-rumped thornbills (1) mob shining bronze-cuckoos, (2) breed earlier than other insectivorous passerine species breeding at similar latitudes, (3) breed earlier than other congeneric species and (4) experience lower parasitism rates if they breed earlier, which would suggest that there is directional selection for early breeding.

METHODS

Study Species and Study Site

Yellow-rumped thornbills are small (9 g), insectivorous, nonmigratory passerines endemic to Australia (del Hoyo, Elliott, Sargata, Christie, & de Juana, 2014). They may breed cooperatively, with the breeding pair receiving assistance in caring for young from one or two nonbreeding helpers, although most published observations of breeding are of pairs (Higgins & Peter, 2002). They breed multiple times during the season, and both sexes participate in building and lining the nest, but only females incubate. They are the primary host of the shining bronze-cuckoo in Australia (Brooker & Brooker, 1989a, 1989b). Shining bronze-cuckoos are breeding migrants, overwintering in Queensland or Papua New Guinea and arriving at our study site in August. The nests of yellow-rumped thornbills are relatively conspicuous, dome-shaped structures with a very small entrance, and may be built in dense clusters of foliage in trees, particularly in mistletoe (Family Loranthaceae) growing on *Eucalyptus* species, or in thorny bushes, such as boxthorn, *Lycium ferocissimum*. We studied yellow-rumped thornbills in Campbell Park, an open eucalypt woodland in Canberra, southeastern Australia (149°9′ E, 35°16′ S), from 1999 to 2015. During our study, nests were located by following adults during nest building or incubation. Nest success was scored as the total number of chicks fledged (with zero for abandoned or depredated nests).

Cuckoo Model Presentation

To test whether yellow-rumped thornbills mob cuckoos, we presented groups of thornbills with freeze-dried specimens of a shining bronze-cuckoo and a nonthreatening control species of similar size that occurs commonly at the study site (white-plumed honeyeater, *Lichenostomus penicillatus*), during two breeding seasons, from August to December in 2012 and 2013. Most of the presentations in both years were done in September, when many of pairs had their second clutch. We used two different specimens each of the shining bronze-cuckoo and the honeyeater and tested for individual specimen effects. The two treatments were presented on the same day, separated by at least 60 min to avoid carryover effects, and the order of presentation was alternated. Trials were conducted during egg laying or early incubation, since this is the period when thornbills are most vulnerable to parasitism. Models were placed inside a fine 1.5 cm wire-mesh cage (50 × 50 cm) to protect them from damage and the cage was placed 2 m from the thornbill nest, at roughly the same height as the nest. The trial commenced when a yellow-rumped thornbill came within 2 m of the model, and continued for 5 min. A video camera (Canon EOS 40D) was positioned at least 10 m from the cage and thornbill responses to the models were filmed. From the video recordings we extracted movements, number of vocalizations in 5 min, and quantified the proportion of time spent within 1 m of the cage. Birds were habituated to the presence of the camera by setting up the camera and tripod from 10 to 15 min before placing the models. This species is very used to anthropogenic intervention, and in several instances birds would continue to mob the model even when the researcher was removing it.

Shifts in Breeding Phenology

To explore whether yellow-rumped thornbills have shifted their breeding phenology in response to brood parasitism, we investigated whether they commence egg laying earlier than other comparable passerine species that breed at similar latitudes in southeastern Australia. Species used for comparison are shown in Appendix Table A1. Like the yellow-rumped thornbill, these are all small insectivorous species and are therefore likely to experience similar constraints on breeding based on food availability. For this analysis, we gathered data on the first month when at least 5% of the eggs were found. We also gathered data on average minimum temperature per month at the Canberra Airport Weather Station (4 km from our field site) from 2008 to 2015, from the Bureau of Meteorology (http://www.bom.gov.au/).

We also explored whether yellow-rumped thornbills in our study site commence egg laying earlier than other *Acanthiza* spp., using data from the Handbook of Australian and New Zealand and Antarctic birds (Higgins & Peter, 2002). We chose egg-laying dates of populations of *Acanthiza* species at similar latitudes or nontropical regions, except for *A. katherina*, which has an exclusively tropical distribution.

To test whether nests of yellow-rumped thornbills that were built earlier in the breeding season had lower parasitism rates than those laid later in the season, we analysed parasitism rates of all yellow-rumped thornbill nests found in Campbell Park in which eggs were laid (cuckoo and/or host eggs), from our long-term data set spanning 1999–2015 (Langmore & Kilner, 2007).

Ethical Note

Our experiment was approved by the Animal experimentation ethics committee at the Australian National University, permit number A2012/30. We did not trap or collect any individual for this study and it was based completely on observations. Nests were accessed to check whether these had eggs but they were only checked once and while the parents were away from the nest.

Statistical Analyses

To test differences in mobbing behaviour in response to the presentation of a cuckoo model versus a honeyeater, we used a generalized linear mixed model (GLMM) in R, with model type (cuckoo or honeyeater), trial order and model replicate as fixed effects and pair ID as random effect. As response variables we used the percentage of time spent less than 1 m from the model and the number of vocalizations. We used a GLMM model with specified Gaussian family distribution, since both response variables were not normally distributed (Time: $W = 0.58$ $P < 0.001$; Calls: $W = 0.49$ $P < 0.001$). To test for an association between egg-laying date and parasitism rate, we used a nominal logistic model, where month of the year was the predictor variable and the response variable was whether or not the nest was parasitized.

RESULTS

The average parasitism rate at our study site was 27.1% of nests in which eggs were laid ($N = 123$ nests, 1999 to 2015), and ranged from 15% to 50% of nests parasitized per season.

Cuckoo Model Presentation

Cooperatively breeding groups were not common at our study site, and all 16 nests used for the experiment belonged to pairs rather than groups. Yellow-rumped thornbills did not show the extremely aggressive response to cuckoo models (Supplementary Video S1) that has been reported in studies of other hosts (e.g. Langmore et al., 2012; Tong et al., 2015; Welbergen & Davies, 2009); but they did spend more time in close proximity to the cuckoo (time spent <1 m from model = 38.2 ± 43%) than the honeyeater (1.9 ± 5.8%; GLMM: $df = 1$, $P = 0.001$; Fig. 1a). The probability of responding to the model did not increase through the season ($X^2 = 0.28$, $P = 0.59$, $N = 16$). Responses were highly dichotomous, and in half of all cuckoo presentations the yellow-rumped thornbill pair made no response. In the other half of trials they attempted to attack the cuckoo through the cage and made vocalizations, but in 87% of cases this response was given by only one member of the pair. In five of the eight cases (63%) of cuckoo attack, we observed that while one individual was close to the cuckoo model, the other individual returned to the nest and sat inside. The vocalizations produced in response to the cuckoo were used in many contexts, and we did not identify any cuckoo-specific type of call, but they gave significantly more vocalizations in response to the cuckoo model than the control (GLMM: $df = 1$, $P = 0.02$; Fig. 1b). There was no effect of the order of presentation ($P = 0.12$) and there was no difference between the responses to the two different models of each species ($P = 0.14$). Given the strikingly different responses to the cuckoo mount from different pairs (attack versus no response), we also examined the number of fledglings produced from the nests used in the experiment for mobbers versus nonmobbers. The number of fledglings produced by parents that mobbed cuckoos was significantly higher than for nonmobbers (ANOVA: $F = 96.33$, $P = 0.0001$; Fig. 1c). Nests of parents that did not mob were either abandoned ($N = 3$), depredated ($N = 3$), naturally parasitized after performing the experiment ($N = 1$) or only fledged partially ($N = 1$), while all nests of parents that mobbed succeeded in fledging young and only one nest had reduced progeny because one egg did not hatch.

Shifts in Breeding Phenology

From 21 species of insectivorous passerines occurring in southeastern Australia for which reliable breeding dates are available (Appendix Table A1), only the yellow-rumped thornbill commences egg laying in July (Fig. 2a). Average minimum temperature in Canberra is lowest during July, with an average minimum temperature of −0.2 °C (Fig. 2a). Yellow-rumped thornbills also lay their eggs before other members of the same genus, *Acanthiza*, which start laying in August and September (Fig. 2b). Yellow-rumped thornbills suffer higher parasitism rates (27.1%) than other thornbill species that breed at the same site, such as the buff-rumped thornbill, *Acanthiza reguloides* (average 11%, $N = 115$ nests, 1999–2014) and the brown thornbill, *Acanthiza pusilla* (average 4%, $N = 23$ nests, 1999–2014). The analysis of egg-laying dates revealed that parasitism rates in yellow-rumped thornbills increase significantly through the season (nominal logistic model: $X^2 = 4.30$, $P = 0.037$, $N = 123$; Fig. 3). Eggs laid in the first 3 months of the breeding season have parasitism rates of around 20%, compared to parasitism rates of 30–50% in the last months of the season (Fig. 3).

DISCUSSION

The yellow-rumped thornbill is the primary host of the shining bronze-cuckoo in Australia, with parasitism rates of up to 50% in some years, yet counter to theoretical expectations, no evidence of defences against cuckoo parasitism has been found in previous studies (Brooker & Brooker, 1989a, 1989b; Langmore et al., 2005). In this study we found evidence that the yellow-rumped thornbill has evolved less explored defence types, such as mobbing behaviour, nest guarding and shifts in breeding phenology.

The mobbing strategy in the yellow-rumped thornbill is much less aggressive than that of other well-studied hosts, such as superb fairy-wrens (Langmore et al., 2012), mockingbirds, *Mimus saturninus* (Gloag et al., 2013) and Cape bulbuls, *Pycnonotus capensis* (Krüger, 2011), in which pairs or groups of birds attack cuckoos fiercely. In our experiments, the cuckoo mount was mobbed in only half the trials, and by only one member of the pair. However, we found that in 63% (5/8) of these trials the other member of the pair returned to the nest. A similar strategy has been reported in carrion crows, *Corvus corone*, and yellow warblers, *Setophaga petechia* (Canestrari, Marcos, & Baglione, 2009; Hobson, 1989), in which the female stays on the nest, discouraging parasite laying attempts.

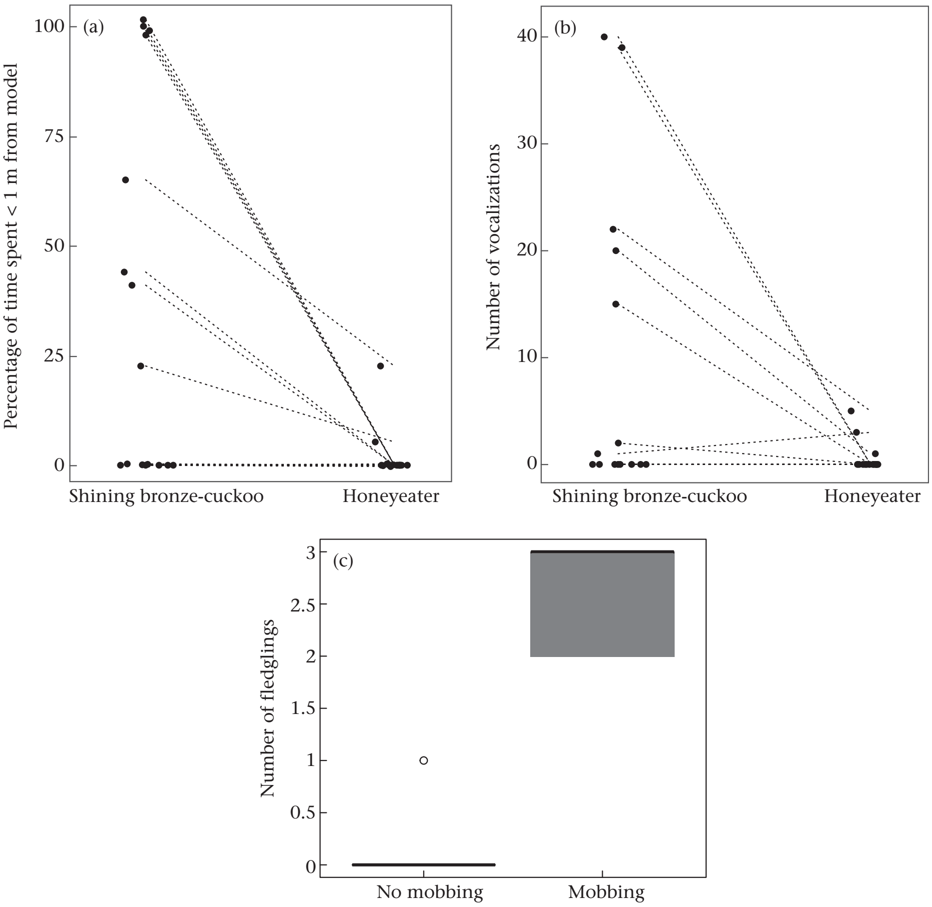


Figure 1. (a) Proximity and (b) vocal response of yellow-rumped thornbills to the cuckoo model and the harmless honeyeater model ($N = 16$ nests). Black lines connect the response of the same individual to both models. (c) Box plot showing number of fledglings produced from nests belonging to individuals that mobbed or did not mob the cuckoo model. The black lines show the median and the box the 25th and 75th percentiles; the circle is an outlier.

This might be an effective defence in yellow-rumped thornbills. Although brood parasites can succeed in laying an egg while the host is on the nest in cup-nesting hosts (e.g. Gloag et al., 2013), this may be more difficult in a dome-shaped nest, where the host may be able to block the small entrance hole. This result highlights the importance of studying defences across various species, in order to detect subtle differences in stereotypical defences, such as mobbing.

Welbergen and Davies (2009) showed a direct link between mobbing and increased reproductive success in reed warblers, *Acrocephalus scirpaceus*, since mobbers tended to have a lower parasitism rate. Here we have shown that pairs with a mobbing response to the cuckoo mount produced more fledglings per nest. In part this may reflect an ability to deflect cuckoos, but it is also likely that both traits are associated with age and experience. A study of another bronze-cuckoo host, the superb fairy-wren, showed that mobbing of cuckoos was learned through observation of mobbing by conspecifics, and did not occur in birds with no prior exposure to cuckoos (Feeney & Langmore, 2013; Langmore et al., 2012). Similarly, great reed warblers, *Acrocephalus arundinaceus*, showed a positive correlation between different behaviours that offer nest protection in general, not only from parasitism (Trnka & Grim, 2014).

In addition to mobbing, we also found evidence that yellow-rumped thornbills may be under selection to adjust their breeding phenology to avoid parasitism. Parasitism rates were lower at the beginning of the season and increased as the breeding season progressed, suggesting that yellow-rumped thornbills may be under directional selection for earlier breeding as a result of parasitism by cuckoos. Correspondingly, this species starts breeding much earlier (mid-winter) than other small passerine species that breed in similar latitudes, and compared to its main parasite the shining bronze-cuckoo (which arrives and commences egg laying in August). In fact, the earliest report of nest building in our field site was 5 June 1996 (Ebert, 2004). Annual average temperatures drop to their lowest point in July (Fig. 2a), which is probably related to low insect abundance and is likely to explain why many other passerines at the site start breeding in mid-August and September. Yellow-rumped thornbills also commence egg laying early relative to congeneric species at our site, such as the buff-rumped thornbill and the brown thornbill, which have much lower parasitism rates, and congeneric species that occur at a

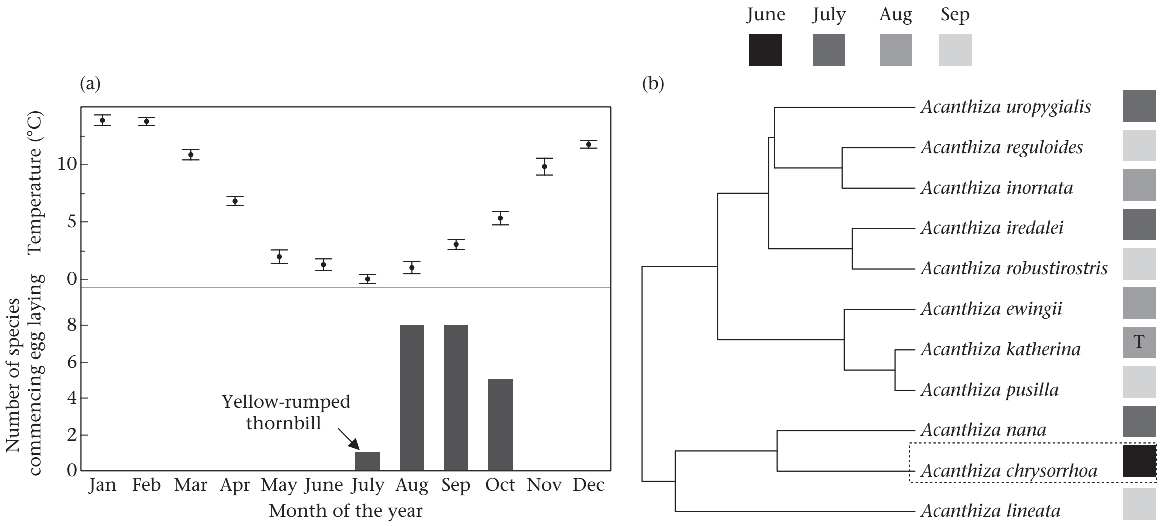


Figure 2. (a) Average minimum temperature ± SD per month in Canberra (2008–2015) and distribution of month of initiation of egg laying for 21 insectivorous passerine species breeding at similar latitudes to our focal species, the yellow-rumped thornbill. (b) Month of commencement of egg laying for species of the genus *Acanthiza*. Data were collected from temperate populations of each species, except for *A. katherina*, which has an exclusively tropical distribution (denoted by **T**).

similar latitude to that of the yellow-rumped thornbills (Fig. 2b). Although these data are correlational and therefore do not prove that shifts in breeding phenology result from brood parasitism pressures, this constitutes one of the first studies explicitly supporting this hypothesis, to our knowledge. A similar suggestion was made for black-capped vireos, *Vireo atricapilla*, in which parasitism rates are lower at the beginning of the breeding season (Boves et al., 2014). Differences in laying dates have also been found for populations of a species of the same family, the grey warbler, *Gerygone igata*, which might be able to avoid shining bronze-cuckoos by laying earlier in the south of their range (Anderson, Gill, Briskie, Brunton, & Hauber, 2013). Similarly, it has been suggested that indigo buntings, *Passerina cyanea*, delay their breeding season to avoid overlap with cowbirds, *Molothrus* spp. (Carey, 1982). It would be interesting to explore the precise mechanisms that have allowed yellow-rumped thornbills to breed in such low temperatures (e.g. nest insulation), and whether this defence strategy is used by other main host species. Moreover, it would be ideal to test whether dates of commencement of breeding in different populations of yellow-rumped thornbills vary according to whether they are sympatric or allopatric with shining bronze-cuckoos, but these data have proven difficult to find, because the breeding ranges of yellow-rumped thornbills and their cuckoos overlap almost completely.

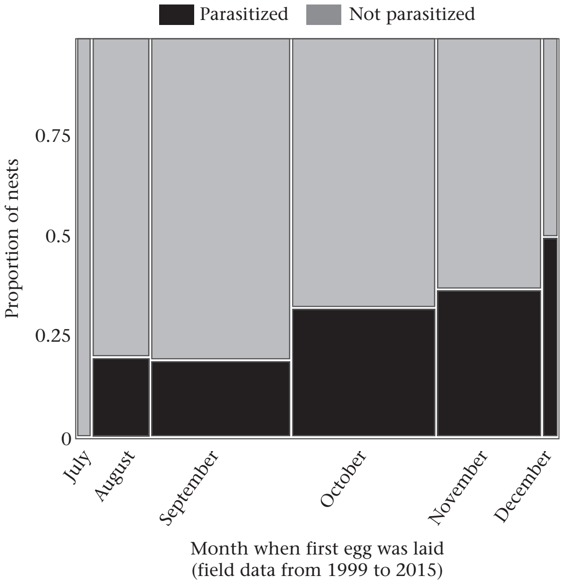


Figure 3. Increase in proportion of parasitized (black) nests of yellow-rumped thornbills through the breeding season. Size of boxes is proportional to sample size per month ($N = 123$ nests).

Although yellow-rumped thornbills are poor egg rejectors, our study suggests that they have evolved at least two types of frontline defences. Our mount experiments show that they can recognize cuckoos, and this may trigger a response in which one member of the breeding pair blocks the entrance of the nest while the other mobs the cuckoo. This mobbing response is linked to the reproductive success of the pair. Moreover, this species also breeds early compared to closely related species and other insectivorous species breeding in southeastern Australia. Our data showed no link between breeding earlier and mobbing less, suggesting that both strategies may be present in the same individual. However, there is evidence that hosts such as superb fairy-wrens can learn to identify parasites (Feeney & Langmore, 2013), so the likelihood of mobbing may increase later in the season, when parasites are more frequent. This is likely to happen in yellow-rumped thornbills but there are no current data. However, the highly dichotomous response of this species is similar to that reported by Davies and Welbergen (2009) in reed warblers, where they suggest that this type of response would be expected in systems in which cuckoo recognition is learned (Davies & Welbergen, 2009). It is also important to mention that the defensive strategies found in this study are not widespread in the population; only 50% of pairs were mobbers and only 3% of the nests were found in July, explaining why this host is still so heavily parasitized. Given our results on reproductive success and parasitism rates through the season, it is possible that these percentages will increase within the next decades. Also, we would expect lower mobbing rates and no shifts in breeding dates in populations with lower parasitism rates.

Finally, we suggest that studying major host species that lack conventional defences, such as group mobbing or egg rejection, might be a fruitful avenue for exploring novel adaptations against brood parasitism. Most species of cowbird hosts and many species with domed nests show low rates of egg rejection (Medina & Langmore, 2015). Rather than being entirely defenceless, these species might employ alternative forms of defence that have been overlooked previously.

Acknowledgments

We thank Ian McConnell-Whalan, T.J. Keyes, Michael Warren, Hazel Speed, Kristal Cain and Wendy Dimond for help finding nests and Andrew Cockburn, Thomas Wallenius and Rob Magrath for comments on the manuscript. We also thank the Canberra Ornithologist Group and Australian Geographic for funding.

Supplementary Material

Supplementary video related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2015.12.006.

References

Anderson, M. G., Gill, B. J., Briskie, J. V., Brunton, D. H., & Hauber, M. E. (2013). Latitudinal differences in the breeding phenology of grey warblers covary with the relationship to the prevalence of parasitism by shining bronze cuckoos. *Emu, 113*(2), 187–191.

Aviles, J. M., Vikan, J. R., Fossoy, F., Antonov, A., Moksnes, A., Roskaft, E., et al. (2012). Egg phenotype matching by cuckoos in relation to discrimination by hosts and climatic conditions. *Proceedings of the Royal Society B: Biological Sciences, 279*(1735), 1967–1976. http://dx.doi.org/10.1098/rspb.2011.2498.

Beaumont, L. J., McAllan, I. A. W., & Hughes, L. (2006). A matter of timing: changes in the first date of arrival and last date of departure of Australian migratory birds. *Global Change Biology, 12*(7), 1339–1354.

Bell, H. L., & Ford, H. A. (1986). A comparison of the social organization of three syntopic species of Australian thornbill, *Acanthiza*. *Behavioural Ecology and Sociobiology, 19*(6), 381–392.

Berry, L. (2001). Breeding biology and nesting success of the eastern yellow robin and the New Holland honeyeater in a southern Victorian woodland. *Emu, 101*(3), 191–197.

Boves, T. J., Sperry, J. H., Comolli, K., & Weatherhead, P. J. (2014). Brood parasitism of black-capped Vireos: frontline and post-laying behavioural responses and effects on productivity. *Journal of Field Ornithology, 85*(4), 364–378.

Bridge, L. (1994). Breeding biology of a migratory population of the rufous whistler *Pachycephala rufiventris*. *Emu, 94*, 106–115.

Brooker, M. G., & Brooker, L. C. (1989a). The comparative breeding behaviour of two sympatric cuckoos, Horsfield's Bronze-Cuckoo *Chrysococcyx basalis* and the Shining Bronze-Cuckoo *C. lucidus*, in Western Australia: a new model for the evolution of egg morphology and host specificity in avian brood parasites. *Ibis, 131*(4), 528–547. http://dx.doi.org/10.1111/j.1474-919X.1989.tb04789.x.

Brooker, M. G., & Brooker, L. C. (1989b). Cuckoo hosts in Australia. *Australian Zoological Reviews, 2*, 1–67.

Canestrari, D., Marcos, J. M., & Baglione, V. (2009). Cooperative breeding in carrion crows reduces the rate of brood parasitism by great spotted cuckoos. *Animal Behaviour, 77*(5), 1337–1344. http://dx.doi.org/10.1016/j.anbehav.2009.02.009.

Carey, M. (1982). An analysis of factors governing pair-bonding period and the onset of laying in Indigo Buntings. *Journal of Field Ornithology, 53*(3), 240–248.

Davies, N. B. (2000). *Cuckoos, cowbirds and other cheats*. London, U.K.: T. & A.D. Poyser.

Davies, N. B. (2011). Cuckoo adaptations: trickery and tuning. *Journal of Zoology, 284*(1), 1–14. http://dx.doi.org/10.1111/j.1469-7998.2011.00810.x.

Davies, N. B., & Welbergen, J. A. (2008). Cuckoo-hawk mimicry? An experimental test. *Proceedings of the Royal Society B: Biological Sciences, 275*, 1817–1822.

Davies, N. B., & Welbergen, J. A. (2009). Social transmission of a host defense against cuckoo parasitism. *Science, 324*, 1318–1320. http://dx.doi.org/10.1126/science.1172227.

Dowling, D. K. (2003). Breeding biology of the red-capped robin. *Australian Journal of Zoology, 51*, 533–549.

Ebert, D. (2004). *Social behaviour and breeding biology of the yellow-rumped thornbill* (Unpunlished doctoral thesis). Canberra, Australia: Australian National University.

Feeney, W. E., & Langmore, N. E. (2013). Social learning of a brood parasite by its host. *Biology Letters, 9*(4), 5.

Feeney, W. E., Medina, I., Somveille, M., Heinsohn, R., Hall, M. L., Mulder, R. A., et al. (2013). Brood parasitism and the evolution of cooperative breeding in birds. *Science, 342*, 1506–1508.

Feeney, W. E., Welbergen, J. A., & Langmore, N. E. (2012). The front-line of avian brood parasite-host coevolution. *Animal Behaviour, 84*(1), 3–12.

Ford, H. A. (1998). Faithfulness to breeding site and birthplace in noisy friarbirds *Philemon corniculatus*. *Emu, 98*, 269–275.

Gardner, J. L. (1998). Experimental evidence for edge-related predation in a fragmented agricultural landscape. *Journal of Ecology, 23*, 311–321.

Gardner, J. L. (2002). Breeding biology of the speckled warbler, *Chthonicola sagittata*. *Australian Journal of Zoology, 50*(2), 169–181.

Gloag, R., Fiorini, V. D., Reboreda, J., & Kacelnik, A. (2013). The wages of violence: mobbing by mockingbirds as a frontline defence against brood-parasitic cowbirds. *Animal Behaviour, 86*(5), 1023–1029.

Green, D. J., & Cockburn, A. (1999). Life history and demography of an uncooperative Australian passerine, the brown thornbill. *Australian Journal of Zoology, 47*(6), 633–649.

Higgins, P. J., & Peter, J. M. (2002). *Handbook of Australian, New Zealand and Antarctic birds*. Melbourne.

Hobson, K. A. (1989). Responses of yellow warblers to the threat of cowbird parasitism. *Animal Behaviour, 38*, 510–519.

del Hoyo, J., Elliott, A., Sargata, J., Christie, D. A., & de Juana, E. (2014). *Handbook of the birds of the world alive*. Barcelona, Spain: Lynx Editions.

Kilner, R. M. (2005). The evolution of virulence in brood parasites. *Ornithological Science, 4*(1), 55–64. http://dx.doi.org/10.2326/osj.4.55.

Krüger, O. (2011). Brood parasitism selects for no defence in a cuckoo host. *Proceedings of the Royal Society B: Biological Sciences, 278*, 2777–2783. http://dx.doi.org/10.1098/rspb.2010.2629.

Krüger, O., Davies, N. B., & Sorenson, M. D. (2007). The evolution of sexual dimorphism in parasitic cuckoos: sexual selection or coevolution? *Proceedings of the Royal Society B: Biological Sciences, 274*, 1553–1560.

Landstrom, M., Langmore, N. E., & Heinsohn, R. G. (2010). Clutch variation and egg rejection in three hosts of the pallid cuckoo, *Cuculus pallidus*. *Behaviour, 147*(1), 19–36. http://dx.doi.org/10.1163/000579509x12483520922043.

Langmore, N. E. (n.d.) [Cuckoo chick rejection dataset for yellow-rumped thornbills]. unpublished raw data.

Langmore, N. E., Feeney, W. E., Crowe-Riddell, J., Luan, H., Louwrens, K. M., & Cockburn, A. (2012). Learned recognition of brood parasitic cuckoos in the superb fairy-wren *Malurus cyaneus*. *Behavioral Ecology, 23*(4), 798–805. http://dx.doi.org/10.1093/beheco/ars033.

Langmore, N. E., Hunt, S., & Kilner, R. M. (2003). Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature, 422*(6928), 157–160. http://dx.doi.org/10.1038/nature01460.

Langmore, N. E., & Kilner, R. M. (2007). Breeding site and host selection by Horsfield's bronze-cuckoos, *Chalcites basalis*. *Animal Behaviour, 74*(4), 995–1004. http://dx.doi.org/10.1016/j.anbehav.2007.02.028.

Langmore, N. E., & Kilner, R. M. (2009). Why do Horsfield's bronze-cuckoo *Chalcites basalis* eggs mimic those of their hosts? *Behavioral Ecology and Sociobiology, 63*(8), 1127–1131. http://dx.doi.org/10.1007/s00265-009-0759-9.

Langmore, N. E., Kilner, R. M., Butchart, S. H. M., Maurer, G., Davies, N. B., Cockburn, A., et al. (2005). The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behavioral Ecology, 6*(4), 686–692. http://dx.doi.org/10.1093/beheco/ari041.

Langmore, N. E., Stevens, M., Maurer, G., Heinsohn, R., Hall, M. L., Peters, A., et al. (2011). Visual mimicry of host nestlings by cuckoos. *Proceedings of the Royal Society B: Biological Sciences, 278*(1717), 2455–2463. http://dx.doi.org/10.1098/rspb.2010.2391.

Magrath, R. D., Leedman, A. W., Gardner, J. L., Giannasca, A., Nathan, A. C., Yezerinac, S. M., et al. (2000). Life in the slow lane: reproductive life history of the white-browed scrubwren, an Australian endemic. *Auk, 117*(2), 479–489.

McComas, T. (1992). *Birds of the Australian Capital Territory: an atlas*. Canberra, Australia: Taylor McComas and Canberra Ornithologists Group.

Medina, I., & Langmore, N. E. (2015). The costs of avian brood parasitism explain variation egg rejection behaviour in hosts. *Biology Letters, 11*(7), 1–5.

Molnár, B. (1944). The cuckoo in the Hungarian Plain. *Aquila, 51*, 100–112.

Moyer, D. C. (1980). On lesser honeyguide and black-collared barbet. *Zambian Ornithological Society Newsletter, 10*, 159.

Munro, K. (2007). Breeding behaviour and ecology of the grey fantail (*Rhipidura albiscapa*). *Australian Journal of Zoology, 55*, 257–265.

Neudorf, D. L., & Sealy, S. G. (1994). Sunrise nest attentiveness in cowbird hosts. *Condor, 96*(1), 162–169.

Noske, R. A. (1998). Social organization and nesting biology of the cooperatively breeding Varied Sittella *Daphoenositta chrysoptera* in north-eastern New south Wales. *Emu, 98*(2), 85–96.

Pell, A. S., & Tidemann, C. R. (1997). The ecology of the common myna in urban nature reserves in the Australian National Territory. *Emu, 97*(2), 141–149.

Radford, J. (2002). *Conservation ecology and breeding biology of the white-browed treecreeper* Climacteris affinis (Unpublished BSc thesis). Melbourne, Australia: University of Melbourne.

Robinson, D. (1989). The nesting ecology of sympatric scarlet robin *Petroica multicolor* and flame robin *P. phoenicea* populations in open eucalypt forest. *Emu, 90*(1), 40–52.

Rowley, I. (1964). The life history of the superb-blue wren *Malurus cyaneus*. *Emu, 64*(4), 251–297.

Sato, N. J., Tokue, K., Noske, R. A., Mikami, O. K., & Ueda, K. (2010). Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. *Biology Letters, 6*(1), 67–69. http://dx.doi.org/10.1098/rsbl.2009.0540.

Spottiswoode, C. N., Stryjewski, K. F., Quader, S., Colebrook-Robjent, J. F., & Sorenson, M. D. (2011). Ancient host specificity within a single species of brood parasitic bird. *Proceedings of the National Academy of Sciences of the United States of America, 108*(43), 17738–17742. http://dx.doi.org/10.1073/pnas.1109630108.

Takasu, F. (1998). Why do all host species not show defense against avian brood parasitism: evolutionary lag or equilibrium? *American Naturalist, 151*, 193–205.

Tokue, K., & Ueda, K. (2010). Mangrove gerygones *Gerygone laevigaster* eject little bronze-cuckoo *Chalcites minutillus* hatchlings from parasitized nests. *Ibis, 152*(4), 835–839. http://dx.doi.org/10.1111/j.1474-919X.2010.01056.x.

Tong, W., Horrocks, N. P. C., & Spottiswoode, C. N. (2015). The sight of an adult brood parasite near the nest is an insuficient cue for a honeyguide host to reject foreign eggs. *Ibis, 157*, 626–630.

Trnka, A., & Grim, T. (2014). Testing for correlations between behaviours in a cuckoo host: why do host defences not covary? *Animal behaviour, 92*, 185–193.

Webster, M. S. (1994). Interspecific brood parasitism of Montezuma oropendolas by giant cowbirds: parasitism or mutualism? *Condor, 96*(3), 794–798.

Welbergen, J. A., & Davies, N. B. (2009). Strategic variation in mobbing as a front line of defense against brood parasitism. *Current Biology, 19*, 235–240.

Welbergen, J. A., & Davies, N. B. (2011). A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behavioral Ecology, 22*(3), 574–579. http://dx.doi.org/10.1093/beheco/arr.

Appendix

Table A1
First month when 5% of the study population laid eggs, for 21 passerine species breeding in southeastern Australia

Species name	Laying commences	Location of study	Reference
Chthonicola sagittata	August	Canberra, ACT	Gardner (2002)
Acanthiza pusilla	August	Canberra, ACT	Green and Cockburn (1999)
Acanthiza reguloides	September	Wollomombi, NSW	Bell and Ford (1986)
Acanthiza lineata	August	Wollomombi, NSW	Bell and Ford (1986)
Sericornis frontalis	August	Canberra, ACT	Magrath et al. (2000)
Malurus cyaneus	August	Canberra, ACT	Rowley (1964)
Daphoenositta chrysoptera	August	Armidale, NSW	Noske (1998)
Acridotheres tristis	October	Canberra, ACT	Pell and Tidemann (1997)
Rhipidura leucophrys	September	Canberra, ACT	Gardner (1998)
Eopsaltria australis	September	Langwarrin, VIC	Berry (2001)
Phylidornyris novaehollandiae	September	Langwarrin, VIC	Berry (2001)
Rhipidura albiscapa	October	Canberra, ACT	Munro (2007)
Philemon corniculatus	August	Armidale, NSW	Ford (1998)
Petroica multicolor	September	Nimmitabel, NSW	Robinson (1989)
Petroica phoenicea	September	Nimmitabel, NSW	Robinson (1989)
Petroica goodenovii	August	Terrick Terrick NP, VIC	Dowling (2003)
Pachycephala rufiventris	October	Armidale, NSW	Bridge (1994)
Climacteris affinis	September	Yarrara NP, VIC	Radford (2002)
Gerygone olivacea	September	Canberra, ACT	Beaumont, McAllan, and Hughes (2006)
Myiagra rubecula	October	Canberra, ACT	Beaumont et al. (2006)
Merops ornatus	October	Canberra, ACT	Beaumont et al. (2006)

Chapter 6

Nest illumination and the evolution of egg rejection in hosts of brood parasites.

This chapter constitutes the second part of my fieldwork in Canberra, Australia.

Nest illumination and the evolution of egg rejection in hosts of brood parasites.

Iliana Medina and Naomi E. Langmore

Abstract

Hosts of avian brood parasites, such as cuckoos, are duped into taking care of a foreign chick and this has led to the evolution of host defences, such as egg rejection. However, many host species are not egg rejecters and it has been suggested that poor illumination inside closed nests may constrain the evolution of this defence. In this study we experimentally increased the light inside the dome nests of yellow-rumped thornbills, the main host of the shining bronze-cuckoo. Contrary to expectations, our results show that rejection events did not increase significantly when nests were brighter, although rejection errors tended to decrease. Moreover, we found that natural light levels inside dome nests were highly variable, and in many cases as high as those in cup-nesting species with high rejection rates. This evidence suggests that, at least currently, egg rejection is not constrained by light levels.

Keywords: Brood parasitism, egg rejection, light, environment, host defences

Introduction

Brood parasites lay their eggs in nests of other species, imposing high costs on their hosts (Davies 2000). The most intensively studied host defence is egg rejection. Many hosts evict brood parasite eggs from the nest, or even abandon the nest and build a new one (Davies 2000). Despite the very high costs of brood parasitism, egg rejection is absent in almost 40% of the species that are considered main hosts (Medina and Langmore 2015). One possibility is that defences may be absent in a population because they have not yet evolved (evolutionary lag hypothesis), but with time and constant selective pressures, these will eventually evolve and spread through the population (Takasu 1998). Alternatively, the 'evolutionary equilibrium' hypothesis states that defences may not evolve in a population if these are more costly than parasitism itself (Brooker and Brooker 1996; Davies 1999). For example, if the host regularly makes recognition errors and rejects its own eggs, the costs of egg rejection may be too high to sustain.

The yellow-rumped thornbill *Acanthiza chrsyorrhoa* is an Australian cuckoo host that rarely rejects foreign eggs (Brooker and Brooker 1989a; Langmore et al. 2005). The lack of egg rejection in this species is puzzling, because it is the primary host of the shining bronze-cuckoo *Chalcites basalis* (Brooker and Brooker 1989b), it suffers high parasitism rates (up to 50% of nests/year, this study), and the costs of parasitism are high; it loses all its own young and invests over a month in care of the cuckoo chick. One possible explanation for the current lack of egg rejection in this species is that egg discrimination is constrained by the dark interior of their dome-shaped nests, but this has not been tested previously (Langmore et al. 2005; Langmore et al. 2009b). Here we test whether low light levels constrain egg rejection in yellow-rumped thornbills, by adding model eggs to both unmanipulated nests and nests that have been modified experimentally to increase illumination in the interior. We predict that if light availability is currently constraining the presence of rejection behaviours in this population, the percentage of eggs rejected will increase in brighter nests and the amount of recognition errors will decrease.

Study species

Yellow-rumped thornbills are small (9 g), insectivorous passerines endemic to Australia. They may breed cooperatively, with the breeding pair receiving assistance in caring for young from one or two non-breeding helpers (Higgins and Peter 2002). They are the primary host of the shining bronze-cuckoo, *Chalcites lucidus*, (Brooker and Brooker 1989a; Brooker and Brooker 1989b). The nests of *A. chrysorrhoa* are relatively conspicuous, dome-shaped structures with a very small entrance (Figure 1A), and may be built in dense clusters of foliage in trees, particularly in mistletoe (Family Loranthaceae) growing on *Eucalyptus* species, or in thorny bushes (e.g. *Lycium* *ferocissimum)*. Their eggs are either immaculate white, or white with fine reddish-brown speckling, and they are quite different from the olive-green eggs of the shining bronze-cuckoo. We studied yellow-rumped thornbills in Campbell Park, an open eucalypt woodland in Canberra, south-eastern Australia (149°9′ E, 35°16′ S), from 1999 to 2015. During our study, nests were located by following adults during nest building or incubation.

Model eggs

We placed a plastic blue model egg in each yellow-rumped thornbill nest. The model eggs matched yellow-rumped thornbill eggs in size (~16 x 12 mm), but differed in colour. We selected bright blue (cobalt turquoise Daler-Rowney acrylic paint) for the models because this is a very conspicuous colour that is very different from that of the host. Models were made of Alumilite Super Plastic cast in silicone moulds. Some species with small bills are unable to grasp an entire brood parasite egg, and instead puncture them to facilitate ejection (Moksnes et al. 1991a). This method of rejection would not be possible with plastic model eggs, so we also used real eggs (N=8) collected from captive zebra-finches (*Taeniopygia guttata,* 15 x 11 mm) or abandoned clutches of superb-fairy wrens (*Malurus cyaneus,* 16 x 12 mm) to test whether rejection rates are higher when puncture-rejection is possible.

Artificial parasitism experiment

To test whether yellow-rumped thornbills can recognize and reject foreign eggs we followed the general procedure of Moksnes et al. (1991a). A single model egg was added to the clutch during egg laying or incubation. The model egg was added without removing any host eggs because egg removal has not been found to influence the probability of model egg rejection in similar experiments (Brooker and Brooker 1989a; Davies and Brooke 1988; Moksnes and Røskaft 1989). The model was left in the nest for five full days and removed on the sixth day if it had not already been ejected. The outcome of the trial was scored as (1) acceptance, if the clutch including the experimental egg was warm and/or the host was incubating or (2) rejection, if the egg had been ejected or buried, or if the nest had been abandoned during this period. We excluded from the analyses nests that were predated during the experiment.

Artificial light experiment

To test whether poor illumination inside nests constrains egg rejection in yellow-rumped thornbills, we increased the light levels of 15 nests experimentally. We gently opened a small window (~ 35 by 35 mm) in the nest material on one side of the top of nest, and wedged a circle of transparent plastic into the window (Figure 1B), allowing the entrance of light but preventing water damage inside the nest or a drastic change in temperature. Illumination inside the nest was measured both before and after creating the window using a Sanwa Illuminance Meter LX2. The narrow probe was inserted into the nest and placed on top of the eggs between 10:00 and noon. Windows were made sufficiently large that the interior of the nest reached at least 35 lux, which is twice the average light we found in nests. We tried to obtain the highest increase in light inside the nest, without dramatically altering the shape of the nest. Thereafter, the protocol followed that of the artificial parasitism experiment above, and we scored the model egg as accepted or rejected after five days.

Figure 1A. Domed nest of yellow-rumped thornbill. **B.** Artificial light experiment with side window covered with transparent plastic.

Results

Artificial parasitism experiment

In unmanipulated nests only two model eggs were rejected (7.69%, *N*=26), and in three nests one of the host's own eggs was evicted (11.54%). No nests were abandoned during the experiment and no eggs showed signs of punctures.

Artificial light experiment

Of the 17 nests used for this experiment, 88% of the birds continued incubating despite the artificial opening made in the nest, however, two of the nests used were depredated before completion of the experiment. After four or five days most pairs closed the artificial window by adding nesting material from the inside. Light inside nests of yellow-rumped thornbills was highly variable between nests, ranging from 0.8 lux up to 64 lux (Figure 2A). The model egg was rejected in 4 out of 15 experiments performed (26.66%), and no hosts evicted their own eggs (Figure 2B). Rejection methods were variable, in one case the blue egg was buried under the nest lining and in the second case the blue egg was found outside the nest. In both cases the parents continued to incubate their own eggs. In the other two cases hosts abandoned the nest after one day and started re-nesting, no extra eggs where laid in the parasitised nest. The four nests where rejection occurred were not the nests with highest light levels (Logistic regression X^2=3.11, P=0.21). There was no significant difference in the number of accepted and rejected model eggs between the natural light and the increased light treatments (Fisher's exact test, P=0.25, N=41, Figure 2B). To confirm these results we performed a power analysis and found that, given our sample size, we had a probability of 85.2% of detecting a large effect (P=0.05). Hence, we don't believe that the non-significant trend we found is due to a low sample size.

The frequency of recognition errors decreased from 60% (2/5) to zero, but the number of egg rejections was so low overall (9 events) that it is difficult to provide robust statistical support for this trend (Fisher's exact test, P=0.19, N=8). Given this number of rejections, the probability of finding a significant result is only 19%.

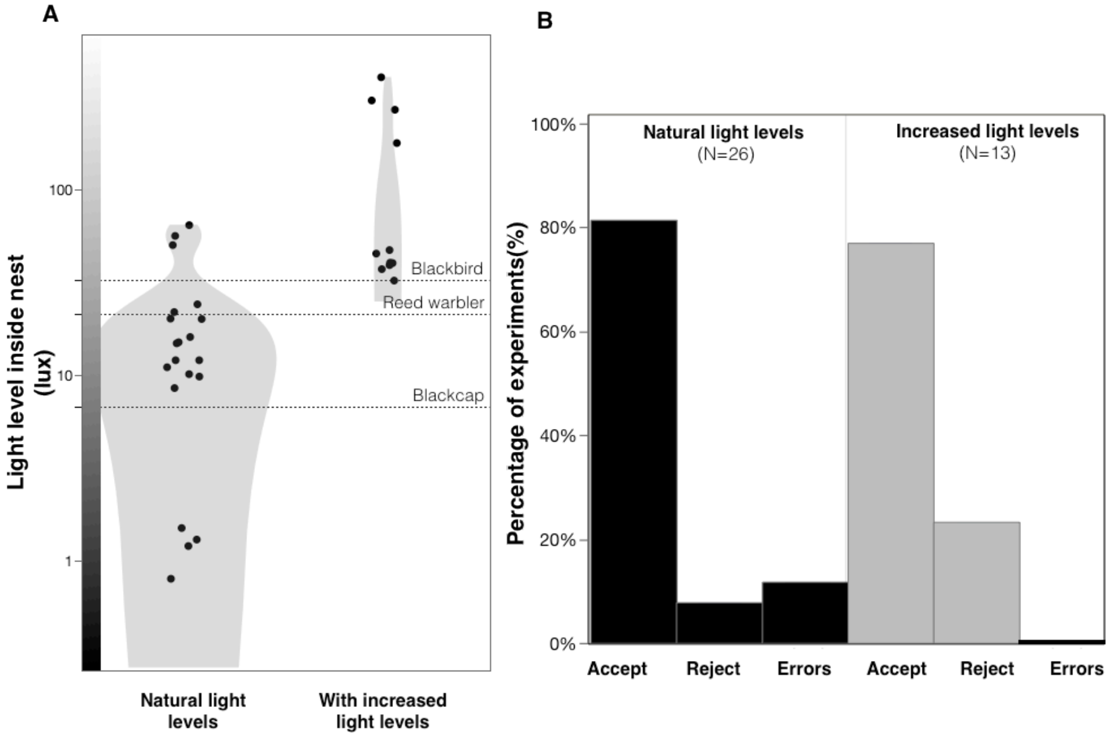


Figure 2. A. Light levels (lux) inside nests of yellow-rumped thornbills with natural light (left, N= 20) and manipulated nests (right, N=13), note the logarithmic scale. Dashed lines indicate light levels for other species with open nests (Data from Langmore et al. 2005). **B.** Proportion of experimental nests in which model eggs were accepted or rejected, or the host rejected one of its own eggs, for unmanipulated (black bars) and manipulated nests (grey bars). There were no significant differences in the frequency of rejection behaviour, although there was a tendency for hosts to make fewer ejection errors in the manipulated nests.

Discussion

We tested the link between egg rejection and light availability by artificially increasing light levels inside the nests of a host species with a domed nest. Our results show that yellow-rumped thornbills are indeed physically capable of evicting eggs from their nests, but the frequency of rejection did not increase in brighter nests. Additionally, we found that they had a moderate risk of making recognition errors. In non-manipulated nests hosts made mistakes by evicting their own eggs instead of the parasitic ones, and rarely succeeded in evicting the foreign egg. The probability of making mistakes decreased from 60% (3/5 rejections) in natural light conditions to zero (0/4) in the increased light treatment. However, there is no statistical support for this trend due to the low number of rejection events.

Light levels varied greatly across nests of yellow-rumped thornbills, and in some nests, light levels were as high as those in open cup nests. For instance, European species such as the blackbird (*Turdus merula*), the blackcap (*Sylvia atricapilla*) and the reed warbler (*Acrocephalus scirparceus*) have open nests with light levels similar to nests of yellow-rumped thornbills (Figure 2A) but the egg rejection rate of these species is much higher (76.92%, 61.82% and 63.89%, respectively, Langmore et al. 2005). Also, the egg rejection events in our experiments did not occur in the nests with the highest light levels. Our findings correspond with a study on the great reed warbler (*Acrocephalus arundinaceus*), a cup nester, and the European magpie (*Pica pica*), which found that there was no correlation between increased ambient light levels and the likelihood of egg rejection (Aviles et al. 2015; Honza et al. 2011). Moreover, our results confirm previous comparative analyses, in which egg rejection rates cannot be explained by differences between closed and open nests (Langmore et al. 2005; Medina and Langmore 2015). Together these studies suggest that currently, poor visibility is not the most important factor behind the absence of egg rejection behaviour. Some studies suggest that an interaction of variables must occur to trigger rejection behaviours, and it is still possible that high light levels increase the likelihood of egg rejection only when combined with other circumstances, such as encountering an adult cuckoo (Langmore et al. 2009a; Soler et al. 2012).

Moreover, it could well be the case that in the past, the difficulty of detecting the dark egg and the high probability of mistakes in dark nests selected against the evolution of egg rejection, and as a result the current population has lost the capacity to recognize and evict parasitic eggs even when light levels are high. Therefore, light levels are currently not related to the absence of egg rejection. It is also possible that such behaviour is constrained by the bird’s genetic makeup, preventing changes within one generation. In any case, our results suggest a low level of phenotypic plasticity in the presence of egg rejection behaviour. It has been shown in magpies that changes in egg rejection behaviour can occur from one breeding season to the other (Molina-Morales et al. 2014), perhaps a longer exposure to experience with bright nests might be necessary before triggering an increase in egg rejection behaviour.

Another explanation for the low rates of egg rejection behaviour in yellow-rumped thornbills could be the 'evolutionary lag' hypothesis, where defences are currently absent but with time will evolve. This explanation seems less likely in our system, because the association between the yellow-rumped thornbill and the shining-bronze cuckoo is likely to be a long-standing one. The earliest record of parasitism of a yellow-rumped thornbill nest by a shining bronze-cuckoo is from 1875 in South Australia, and the earliest record from out study site is at least 60 years old, (egg collection, Australian National Wildlife Collection, CSIRO). Theoretical models predict that under high parasitism rates, such as those experienced by yellow-rumped thornbills, a rejecter allele should spread through 60% of the population in about 60 years (Takasu et al. 1993). Empirical evidence in support of this prediction is provided by a study of the azure-winged magpie *Cyanopica cyanus*, which expressed high rates of egg rejection within 20 years of exposure to parasitism by the common cuckoo *Cuculus canorus* (Nakamura 1990). Thus evolutionary lag seems an unlikely explanation for the low rates of egg rejection in our system.

The 'strategy blocking' hypothesis proposes that the evolution of an effective front-line of defence, such as mobbing, would reduce selection for the evolution of further defences such as egg rejection (Britton et al. 2007). This hypothesis was inspired by the fact that the vast majority of species that recognize and reject parasitic chicks are unable to reject parasitic eggs (Grim 2006), suggesting a trade-off between the two defences. Nevertheless, there is also evidence that different types of defences can evolve in the same population. Moksnes et al. (1991b) and Guigueno and Sealy (2011) found a positive relationship between the presence of egg rejection and cuckoo mobbing for hosts of the European cuckoo (*Cuculus canorus*) and the brown-headed cowbird (*Molothrus ater*), respectively. There is recent evidence that yellow-rumped thornbills can recognize and mob their parasite, and avoid parasitism by breeding early (Medina and Langmore, in press), which supports the idea that front-line defences are hindering the evolution of egg-rejection. However, neither of these defences are sufficiently effective to prevent yellow-rumped thornbills from suffering high rates of parasitism, so strategy blocking does not appear to apply in this case.

In conclusion, we show that nest light levels can vary greatly within a species, even in enclosed nests. An experimental increase in the illumination of nests did not increase the probability of egg-rejection. We suggest that, currently, light availability may not be a determining factor explaining the absence of egg rejection in this species, but it may help explain the occurrence of mistakes in egg-rejection.

References

Aviles, J.M., Martin-Galvez, D., De Neve, L., Soler, M. & Soler, J.J. (2015). Ambient light in domed nests and discrimination of foreign egg colors. *Behavioural Ecology and Socibiology*, 69, 425-435.

Britton, N., R. Planqué, and N. Franks. 2007. Evolution of defence portfolios in exploiter–victim systems. Bulletin of Mathematical Biology 69:957-988.

Brooker, M., and L. Brooker. 1996. Acceptance by the splendid fairy-wren of parasitism by Horsfield's bronze-cuckoo: further evidence for evolutionary equilibrium in brood parasitism. Behavioral Ecology 7:395-407.

Brooker, M. G., and L. C. Brooker. 1989a. The comparative breeding behaviour of two sympatric cuckoos, Horsfield's Bronze-Cuckoo *Chrysococcyx basalis* and the Shining Bronze-Cuckoo *C. lucidus*, in Western Australia: a new model for the evolution of egg morphology and host specificity in avian brood parasites. Ibis 131:528-547.

Brooker, M. G., and L. C. Brooker. 1989b. Cuckoo hosts in Australia. Australian Zoological Reviews 2:1-67.

Davies, N. B. 1999. Cuckoos and cowbirds versus hosts: Co-evolutionary lag and equilibrium. Ostrich: Journal of African Ornithology 70:71 - 79.

Davies, N. B. 2000. Cuckoos, cowbirds and other cheats. London, T. & A.D. Poyser.

Davies, N. B., and M. d. L. Brooke. 1988. Cuckoos versus reed warblers: adaptations and counteradaptations. Animal Behaviour 36:262-284.

Grim, T. 2006. The evolution of nestling discrimination by hosts of parasitic birds: why is rejection so rare? Evol. Ecol. Res. 8:785-802.

Guigueno, M. F., and S. G. Sealy. 2011. Aggression towards egg-removing cowbird elicits clutch abandonment in parasitized yellow warblers, *Dendroica petechia*. Animal Behaviour 81:211-218.

Higgins, P. J., and J. M. Peter. 2002, Handbook of Australian, New Zealand and Antartic Birds. Melbourne.

Honza, M., P. Prochazka, K. Morongova, M. Capek, and V. Jelinek. 2011. Do nest light conditions affect rejection of parasitic eggs? A test of the light environment hypothesis. Ethology 117:7.

Honza, M., M. Sule, and M. I. Cherry. 2014. Does nest luminosity play a role in recognition of parasitic eggs in domed nests? A case study of the red bishop. Naturwissenschaften 101:1009-1015.

Langmore, N. E., A. Cockburn, A. F. Russell, and R. M. Kilner. 2009a. Flexible cuckoo chick-rejection rules in the superb fairy-wren. Behavioural Ecology 20:978-984.

Langmore, N. E., R. M. Kilner, S. H. M. Butchart, G. Maurer, N. B. Davies, A. Cockburn, N. A. Macgregor et al. 2005. The evolution of egg rejection by cuckoo hosts in Australia and Europe. Behavioral Ecology 6:686-692.

Langmore, N. E., M. Stevens, G. Maurer, and R. M. Kilner. 2009b. Are dark cuckoo eggs cryptic in host nests? Animal Behaviour 78:461-468.

Medina, I., and N. E. Langmore. 2015. The costs of avian brood parasitism explain variation egg rejection behaviour in hosts. Biology Letters 11:1-5.

Medina, I., and N.E. Langmore. 2016. Batten down the thatches: Front-line defences in an apparently defenceless cuckoo host. Animal behaviour 112: 195-201.

Moksnes, A., and E. Røskaft. 1989. Adaptations of meadow pipits to parasitism by the common cuckoo. Behavioral Ecology and Sociobiology 24:25-30.

Moksnes, A., E. Røskaft, and A. T. Braa. 1991a. Rejection Behavior by Common Cuckoo Hosts Towards Artificial Brood Parasite Eggs. The Auk 108:348-354.

Moksnes, A., E. Skaft, A. T. Braa, L. Korsnes, H. M. Lampe, and H. C. Pedersen. 1991b. Behavioural Responses of Potential Hosts Towards Artificial Cuckoo Eggs and Dummies. Behaviour 116:64-89.

Molina-Morales, M., Martinez, J.G., Martin-Galvez, D., Dawson, D.A., Burke, T. & Avilés, J.M. (2014). Cuckoo hosts shift from accepting to rejecting parasitic eggs across their lifetime. *Evolution*, 68, 3020-3029.

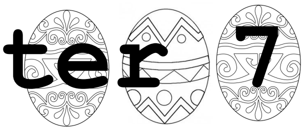
Nakamura, H. 1990. Brood parasitism by the cuckoo *Cuculus canorus* in Japan and the start of new parasitism on the Azure-winged magpie *Cyanopica cyana*. Japanese journal of ornithology 39:1-18.

Soler, M., M. Martin-Vivaldi, and J. Fernandez-Morante. 2012. Conditional response by hosts to parasitic eggs: the extreme case of the rufous-tailed scrub robin. Animal behaviour 84:421-426.

Takasu, F. 1998. Why do all host species not show defense against avian brood parasitism: Evolutionary Lag or Equilibrium? The American Naturalist 151:193-205.

Takasu, F., K. Kawasaki, H. Nakamura, J. E. Cohen, and N. Shigesada. 1993. Modeling the Population Dynamics of a Cuckoo-Host Association and the Evolution of Host Defenses. The American Naturalist 142:819-839.

Chapter 7



Brood parasitism is linked to egg pattern diversity within and among species of Australian passerines.

This project was performed in collaboration with researchers from the University of Exeter, and is a product of my visit to the United Kingdom. This paper was published on 2016 in *The American Naturalist*.

VOL. 187, NO. 3 THE AMERICAN NATURALIST MARCH 2016

Brood Parasitism Is Linked to Egg Pattern Diversity within and among Species of Australian Passerines

Iliana Medina,[1,*] Jolyon Troscianko,[2] Martin Stevens,[2] and Naomi E. Langmore[1]

1. Department of Ecology, Evolution, and Genetics, Australian National University, Canberra, Australia; 2. Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall TR10 9FE, United Kingdom

Submitted February 25, 2015; Accepted September 18, 2015; Electronically published February 3, 2016

Online enhancements: appendix. *Dryad data:* http://dx.doi.org/10.5061/dryad.s31sp.

ABSTRACT: Bird eggs show striking diversity in color and pattern. One explanation for this is that interactions between avian brood parasites and their hosts drive egg phenotype evolution. Brood parasites lay their eggs in the nests of other species, their hosts. Many hosts defend their nests against parasitism by rejecting foreign eggs, which selects for parasite eggs that mimic those of the host. In theory, this may in turn select for changes in host egg phenotypes over time to facilitate discrimination of parasite eggs. Here, we test for the first time whether parasitism by brood parasites has led to increased divergence in egg phenotype among host species. Using Australian host and nonhost species and objective measures of egg color and pattern, we show that (i) hosts of brood parasites have higher within-species variation in egg pattern than nonhosts, supporting previous findings in other systems, and (ii) host species have diverged more in their egg patterns than nonhost species after controlling for divergence time. Overall, our results suggest that brood parasitism has played a significant role in the evolution of egg diversity and that these effects are evident, not only within species, but also among species.

Keywords: diversity, egg phenotype, brood parasitism, variability, diversity, Cuculidae.

Introduction

Explaining phenotypic diversity is one of the main challenges of evolutionary biology. To understand observed diversity patterns, it is important to identify the mechanisms that underlie phenotypic variation within species and then study these in a broader context with the use of phylogenetic methods. For instance, egg phenotype in birds is impressively diverse, and such variability is unevenly distributed across the avian phylogeny; some families of birds produce immaculate white eggs exclusively, whereas others show dramatic variability between and even within species (Kilner 2006). Earlier studies have proposed thermoregulation and camouflage as possible explanations for this variability (Westmoreland et al. 2007; Mayer et al. 2009), but another interesting hypothesis that has not been explicitly tested is that coevolution between avian interspecific brood parasites and their hosts might drive increased diversity in egg phenotypes among species (Kilner 2006).

Avian brood parasites lay their eggs in the nests of other species (their hosts). This behavior has evolved independently seven times in the avian phylogeny, and the largest radiation has occurred in the family Cuculidae (~40 species; Payne and Payne 1998). Parasitism typically results in the loss of host young, and the host parents then invest many weeks rearing the imposter chick, often reducing opportunities for renesting within the season (Davies 2000). This behavior is so costly to the host that it has led to the evolution of multiple defenses against parasitism, including mobbing of adult brood parasites (Welbergen and Davies 2009; Feeney et al. 2012; Langmore et al. 2012) and rejection of foreign eggs (Rothstein 1975; Moksnes et al. 1991; Avilés et al. 2004; Spottiswoode and Stevens 2010), chicks (Grim 2007; Langmore et al. 2003; Sato et al. 2010), or fledglings (de Mársico et al. 2012).

Rejection of foreign eggs by hosts is one of the most widespread defenses against brood parasitism. In empirical studies, 63.3% of commonly exploited species showed egg rejection (i.e., they effectively reject >90% of foreign eggs; Soler 2014). The evolution of egg rejection has led to an arms race; egg rejection by hosts selects for brood parasite eggs that resemble those of the host (Stoddard and Stevens 2010, 2011), which in turn selects for changes to the host egg phenotype that facilitate discrimination of foreign eggs (Davies 2000).

Brood parasitism has proved to be a particularly important driver of variation in egg phenotype within species (Stokke et al. 2002; Underwood and Sealy 2002; Kilner 2006; Spottiswoode and Stevens 2012). Host species can evolve different strategies to increase discrimination abilities and facilitate the detection of a parasitic egg. Theory predicts that (i) hosts should evolve eggs that are individually distinct from those of other females (e.g., high within-species variation), which decreases the likelihood that a parasite's eggs will

---

[*] Corresponding author; e-mail: iliana.medina@anu.edu.au.

Am. Nat. 2016. Vol. 187, pp. 000–000.
DOI: 10.1086/684627

match their own eggs, and (ii) hosts should evolve reduced within-clutch variation, because uniformity should facilitate discrimination of a foreign egg (Davies and Brooke 1989*a*; Langmore and Spottiswoode 2012). The first hypothesis has been well supported by comparing within-population egg variation in host populations that are allopatric and sympatric with brood parasites (Avilés and Møller 2003; Lahti 2005) and in comparative analyses (Soler and Møller 1995; Stokke et al. 2002). Moreover, individuals within the same host species may evolve distinctive signature patterns of blotches and markings (Swynnerton 1918; Victoria 1972; Stoddard et al. 2014; Caves et al. 2015), which makes their eggs highly recognizable. The second hypothesis has received mixed support. Some studies have found greater within-clutch egg uniformity in hosts than nonhosts (Avilés and Møller 2003; Moskát et al. 2008), but many others have failed to find support for the prediction (Avilés et al. 2004; Stokke et al. 2004; Cherry et al. 2007; Landstrom et al. 2010; Stoddard et al. 2014). Discrimination can be achieved by the use of just one of the strategies mentioned above; for example, species with high within-clutch variation can still have highly recognizable eggs if these have distinctive markings (Stoddard et al. 2014). Indeed, a recent study used image analysis and calculations of "entropy" of the egg markings of hosts and nonhosts of two African parasitic species, showing that host egg color and marking components have lower levels of correlation with one another than do those of nonhosts, thus potentially affording greater information about egg identity (Caves et al. 2015).

Many studies have supported the predictions stated above within various host species (Soler and Møller 1995; Stokke et al. 2002; Spottiswoode and Stevens 2011; Stoddard et al. 2014; Caves et al. 2015), suggesting that variation in egg color and pattern within species may be a response to brood parasitism. But can brood parasitism also be associated with egg diversity among species? If there is selection for novel phenotypes within host species (e.g., to have particularly distinguishable patterns, which facilitate discrimination of parasite eggs), and host eggs are selected to occupy different regions in the phenotypic space, variation among host species may arise as a byproduct of selection (fig. 1). The scenario described above leads to the prediction that, in the same amount of evolutionary time, two host species that are subject to brood parasitism will evolve more differences in egg phenotype between them than two nonhost species. Alternatively, increased constraints on the evolution of egg phenotype of hosts could result in decreased diversity of egg phenotypes among host species. The hypothesis that brood parasitism is associated with increased egg diversity among species is somewhat supported by an analysis performed at high taxonomic levels, where Kilner (2006) found a slight, nonsignificant association between high egg diversity

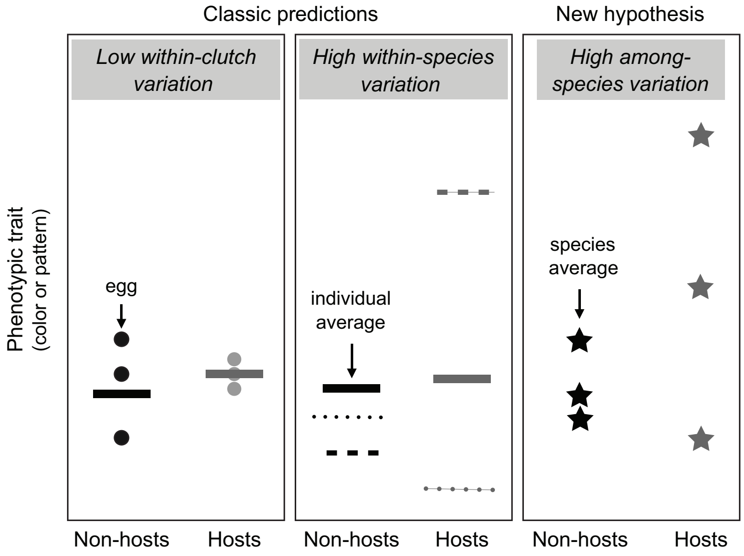


Figure 1: Illustration of the three hypotheses tested in the article. The *Y*-axis represents the values for any egg phenotypic trait (color or pattern). *Left*, points correspond to the value for each egg within a clutch. *Middle*, the different lines represent averages per individual in their egg phenotype. *Right*, the stars represent the average egg phenotype for a species, and hosts are predicted to have higher egg phenotypic variation among species than nonhosts.

between species within a family and the level of exploitation by interspecific brood parasites. However, this study was based on family-level information and a data set that did not use objective measures of color and pattern. Since then, there have been well-resolved bird phylogenies and significant technological advances that allow quantification of color and pattern as seen through the eyes of a bird (Spottiswoode and Stevens 2010; Stoddard and Stevens 2011).

In our study, we use reflectance spectrometry, digital pattern analyses, and phylogenetic information to test whether brood parasitism is associated with higher egg diversity between host species. We used species of Australian hosts and nonhosts together with their phylogenetic relationships to test whether host species have evolved greater differences in egg phenotype than nonhost species, controlling for evolutionary time. Moreover, because our data set includes hosts of six different brood parasite species, we explored whether species exploited by the same parasite evolve more or fewer differences between them than host species parasitized by different species.

Australian brood parasites (cuckoos) and their hosts are a relatively unknown study system compared with those of Europe or North America, where the classic predictions associated with within-species variability have already been verified in comparative analyses (e.g., Soler and Møller 1995; Stokke et al. 2002). The diversity of hosts (main hosts = 90 species) and cuckoo species ($n = 10$) breeding in Australia make this an ideal system for testing the two classic predictions associated with clutch variation; whether hosts have (i) low within-clutch variation and (ii) high within-species variation in egg morphology (fig. 1). We also use the Australian system to conduct the first test of the hypothesis that host species have evolved greater diversity in their egg phenotypes than closely related nonhosts (fig. 1). Eight of the 10 Australian parasitic cuckoos lay eggs that closely resemble those of their primary hosts (Brooker and Brooker 1989; Beruldsen 2003; Starling et al. 2006; Feeney et al. 2014), whereas two bronze-cuckoo species that parasitize dome-nesting hosts have evolved egg crypsis rather than mimicry (Langmore et al. 2009; Gloag et al. 2014). There is evidence of polymorphic, host-specific egg types in two cuckoo species, the Pallid cuckoo, *Cacomantis pallidus*, and the Brush cuckoo, *Cacomantis variolosus* (Beruldsen et al. 2003; Starling et al. 2006; Langmore et al. 2009). Like the majority of cuckoo hosts elsewhere, most cup-nesting host species in Australia show high rates of egg rejection (77%; table A1, available online), suggesting that the morphology of host eggs may be under selection to facilitate accurate egg discrimination by hosts. Even among dome-nesting hosts, which show lower rates of egg rejection (12.66%; table A1), egg morphology may be under selection as a result of brood parasitism, because the cuckoo removes a single egg during parasitism and is more likely to remove an egg with high luminance (Gloag et al. 2014), which may select for dark pigment in host eggs. Moreover, cuckoos in Australia are highly virulent, evicting all host eggs or outcompeting all host nestlings in the nest (Brooker and Brooker 1989). This results in higher costs of parasitism than for many hosts of nonevicting parasites (such as cowbirds *Molothrus* species and the greater spotted cuckoo *Clamator glandarius*), providing stronger selection for the evolution of defenses (Kilner 2005).

Methods

Data Collection

We took photographs and spectral data for 517 eggs in 200 clutches from 40 Australian passerine species (22 hosts and 18 nonhost species) belonging to 25 different genera (fig. A1; figs. A1–A4 available online) at the Australian National Wildlife Collection egg collection in Canberra (ACT). We measured three eggs per clutch (or fewer for species that lay smaller clutches) and five clutches per species, and we confirmed that the clutches were similarly distributed in time and space for hosts and nonhosts (fig. A2). Photographs were taken for egg pattern analyses, and spectral data were collected for color analyses (see below). For our analysis, we included some of the passerine species classified by Brooker and Brooker (1989) as either nonhosts ($n = 18$) or biological main hosts ($n = 22$) of Australian cuckoos. The species we used were selected from the Brooker and Brooker (1989) data set to maximize the number of phylogenetically independent lineages present in the sample while keeping a balance between the number of host and nonhost species. For instance, wherever there was a phylogenetically independent origin for host status (host or nonhost), we sampled that clade and its sister clade. Also, almost all of the thornbill and honeyeater species are parasitized; therefore, to avoid a bias in the analyses toward particularly large clades, we arbitrarily sampled four or five species as representatives of the clade. We collected data on host status from Brooker and Brooker (1989). They applied rigorous criteria to their classification of hosts as biological (successful) hosts, distinct from accidental or unsuitable hosts; biological hosts had multiple (>4) independent (>1 observer, >1 location, >1 year) records of parasitism, and either (i) an egg or authentic nestling record followed through to fledgling; (ii) nestling and feeding records as well as egg records; or (iii) egg records alone, if congeneric with a known biological host of the cuckoo. We excluded species classified as nonbiological (occasional or rare) hosts, because it was not possible to infer the extent of selection on these hosts by brood parasites. We also collected data on nest type from the *Handbook of the Birds of the World Alive* (del Hoyo et al. 2014), given the evidence showing that egg rejection is less likely in enclosed nests than in open cup-shaped nests (Langmore et al. 2005). In our data set, 40% of the species were dome nesters.

Egg Pattern Analyses

We took photographs of each egg with a Canon EOS 50D camera and a 100-mm f/2.8 Macro lens. We included a 16% gray standard and a 1-cm scale in each of the photographs. All image analyses were performed in ImageJ (Rasband 2014), using custom-written code. The green (medium wavelength) channel was extracted from each photograph and used for pattern analysis, following Spottiswoode and Stevens (2010). This approximates to an achromatic luminance channel of avian vision, with achromatic information widely thought to be most important in pattern processing in animal vision (Osorio and Vorobyev 2005). All photographs were taken in the same laboratory and under similar light conditions, but to further standardize for different lighting conditions, each image was also linearized (fit $R^2 = 0.999$) and converted to reflectance relative to the gray standard (Stevens et al. 2007). Images were scaled to 45 pixels per millimeter, and eggs were selected using an egg-shape selection tool (Troscianko 2014). Pattern analysis was then performed using fast Fourier transform bandpass filtering at different spatial scales from 2 pixels increasing exponentially with $\sqrt{2}$ to 512 pixels. This type of "granularity" pattern analysis has been used in a number of previous studies to analyze animal markings (e.g., Godfrey et al. 1987; Stoddard and Stevens 2010). The granularity filtering approach is broadly based on well-established principles of lower-level vision, including receptive fields and spatial frequency filtering, and is supported by the neurophysiology of a range of vertebrate and invertebrate animal species (Campbell and Robson 1968; Godfrey et al. 1987; Stoddard and Stevens 2010). In addition, granularity-based metrics have been tried and tested with several field experimental studies of egg rejection, showing that the pattern metrics derived do predict rejection behavior (e.g., Spottiswoode and Stevens 2010, 2012; Stevens et al. 2013). We therefore chose this method over a recent feature-detection-based approach (Stoddard et al. 2014), which used techniques developed for machine vision and object recognition, because there is little clear evidence that the latter approach approximates to how object and pattern recognition work in animals. In addition, the machine-learning approach is yet to be validated with behavioral egg-rejection experiments, and so we cannot at present say with confidence whether the measures derived from it are relevant to how birds reject foreign eggs.

The amount of "energy" contained in each pattern was measured using a histogram at each spatial scale as the standard deviation of the pixel intensities. The resulting granularity spectra were used to generate descriptive statistics of each egg pattern, including marking diversity (the proportion of the total energy accounted for by the dominant marking size; i.e., the higher the value, the more one marking size dominates), contrast of the patterns against the background (total energy or amplitude of the spectrum), and dominant marking size (denoted by peak frequency of the spectrum; Stoddard and Stevens 2010).

Pattern Differences between Species

Pattern difference between two species was calculated as the sum of the absolute differences in the species average energy at each spatial scale (i.e., the overall differences in the granularity spectra, taking into account both shape and amplitude). A matrix of between-species pairwise differences in pattern was generated. This measurement describes pattern similarity in a manner that, unlike the descriptive statistics, can compare multimodal energy spectra that have more than one peak frequency, preserving all pattern information and combining across all spatial scales measured.

Pattern Variation within Species and within Clutches

To calculate the degree of polymorphism within each species and within individuals, we used the mean contrast, mean dominant marking size, and mean marking diversity values from the pattern analysis for each photograph. We then calculated the standard deviation within each clutch (within-clutch variation) and used the average value per species. To calculate within-species variation in egg pattern, we calculated the standard deviation within each species using the same variables described above.

Color Analyses

We measured reflectance spectra of egg background color at three different places on the egg (base, middle, and tip) with a 5-mm-diameter probe and measured speckle color with a smaller probe (3-mm diameter) using an Ocean Optics USB4000 spectrometer. The visual systems of birds can be divided into two discrete classes; those with retinal pigments sensitive to shorter wavelength UV light (UVS group) and those with pigments sensitive to longer wavelength violet light (VS group; Cuthill 2006). Most passerines have UVS-type cones, but shifts between visual systems can occur even within a single genus (Ödeen et al. 2012). Therefore, to assess egg color and pattern as seen through the eye of a bird, we calculated photon catches for the visual systems of both the blue tit *(Cyanistes caeruleus)* and the common peafowl (*Pavo cristatus*), which are commonly used models of a UVS and VS system, respectively (cone ratios UVS 1 : 0.99 : 0.71 : 0.37 and VS 1 : 1.9 : 2.2 : 2.1; Hart and Hunt 2007). Spectral sensitivity data are available for very few bird species (Hart 2001), so most studies use the visual systems of the blue tit *Cyanistes caeruleus* and the peafowl *Pavo cristatus* as models of UVS and VS visual systems, respectively (e.g., Avilés et al. 2010; Stoddard and Stevens 2010; Spottiswoode and Stevens 2012). These model species are distantly related, so they reveal the extent to which the results vary depending on the visual system used. We used a linear mixed model in R to compare the differences between visual systems; we report the F statistic and the P value.

Photon catch values were used in a model that predicts discrimination abilities for color and yields a value of just noticeable differences (JNDs). We used the log version of the standard Vorobyeb and Osorio model (1998) using Weber fractions of 0.05 or 0.02 where a JND of less than 1.00 means two objects are not distinguishable, and discrimination is unlikely when values are under 3 JNDs (Siddiqi et al. 2004). We generated a pairwise distance matrix of JNDs in color between all the species, for both visual systems. As in the case of pattern analysis, this pairwise approach is much more accurate than reducing variables to give color values per species. To calculate color polymorphism, we used the same procedures described in the pattern analyses using values for each cone.

New Hypothesis: Diversity of Egg Color and Pattern among Species

To test whether, on average, host species evolve more differences in egg phenotype than nonhost species, we made pairwise comparisons within two sets: among host species and among nonhost species. Phenotypic differences between species are expected to increase with time (Martins 1994); therefore, to make objective comparisons between host and nonhost pairs, we had to include information about the time of divergence between each pair of species. To do so, we downloaded 1,000 different possible phylogenetic trees from a pseudoposterior distribution from birdtree.org (Jetz et al. 2012). By doing the same analyses across different phylogenetic hypotheses, we made sure that our results were independent from the tree used. From each tree, we generated matrices with phylogenetic distances between species using the cophenetic.dist command in the R package ape (Paradis et al. 2004). Because the trees were time calibrated, distances are directly proportional to time (millions of years ago; MYA) and can actually be interpreted as such.

Because we were interested in finding out whether pairs of host species would diverge more than pairs of nonhost species, we included another variable called “type of comparison.” This variable refers to the fact that some phenotypic distances were calculated between host species (host vs. host) and others were calculated between nonhost species (nonhost vs. nonhost). Thus, if hosts were more diverse, we would predict larger phenotypic distances in comparisons between host species than between nonhost species.

We used general linear models (Legendre and Fortin 2010) to test our hypothesis, and we included the following as predictors: phylogenetic distance, type of comparison, and phylogenetic distance × type of comparison. As response variables, we included egg color differences (JNDs) and egg pattern differences. This last variable was transformed using natural logarithm to achieve normality. We report the average P value and average β across the 1,000 trees for each predictor in the model. We also report the standard deviation for these values.

If phylogenetic distance predicts differences in color and pattern, it suggests that related species are more similar to each other than nonrelated species. If the type of comparison effectively predicts differences in color and pattern, it suggests that being a host or a nonhost species is associated with the degree of diversity in color and pattern, despite phylogenetic relatedness. If the interaction between both variables predicts differences in color and pattern, it suggests that color and pattern differences evolve differently in hosts and nonhosts (the slopes are different). Additionally, to explore whether host species that shared the same parasite evolved more or fewer phenotypic differences, we included a variable that coded whether the pair of host species shared the same parasite or a different parasite. It is worth pointing out that the rationale behind all this analysis is somewhat analogous to measuring evolutionary rates; if a pair X of species evolves more phenotypic differences than pair Y in the same time, it suggests that pair X evolved faster. We did not use specific methods to measure evolutionary rates for two reasons. First, current methods to measure pattern and color differences are much more accurate when doing pairwise comparisons than when summarizing information in a one-dimensional variable. Second, several nonhost species are contained within clades of hosts, and current phylogenetic methods are designed to calculate and compare rates between clades, not among singular branches.

Classic Predictions: Variation in Egg Pattern within Species and within Clutches

To test whether host eggs are more or less polymorphic than nonhosts, we used a phylogenetic linear regression (pgls) in the Caper package in R (Orme et al. 2012). We used host status and nest type as predictor variables and within-clutch and within-species variation in color and pattern as response variables. Nonsignificant predictors were dropped one at a time until we obtained a model with only significant predictors. We report the P value, β, and λ (e.g., phylogenetic signal, when $\lambda = 0$ the relationship between predictor and response is unaffected by phylogeny). The analyses were repeated for 1,000 different trees obtained from birdtree.org

(Jetz et al. 2012). The data set with final pattern and color measurements has been deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.s31sp (Medina et al. 2016).

Results

Eggs of both hosts and nonhosts varied from plain white eggs, such as those of some thornbills (*Acanthiza* species) and finches (*Poephila* species), to speckled and darker eggs, such those of fantails (*Rhipidura* species) and lyrebirds (*Menura* species). There were no significant differences in color or pattern between hosts and nonhosts (pgls, color: $P > .5$ for all the cones in both background and speckles; pattern: proportion energy $P = .40$, peak frequency $P = .18$, total energy $P = .23$). In our sample, we found no association between being a host and having a closed nest; the distribution of closed versus open nests was 40% for hosts and 38% for nonhosts, and nest type had no significant effect in any of the analyses reported below.

Differences between Visual Systems

Differences in color (JNDs) between pairs of species were significantly higher when the data were analyzed with the blue tit visual system than with the peafowl system (fig. A3). This was true for both background (BG) and speckle color (SP; linear mixed model, BG: for host vs. host, $F = 150.34$, $P < .0001$, df = 228, 459; for nonhost vs. nonhost, $F = 91.00$, $P < .0001$, df = 180, 303; SP: for host vs. host, $F = 244.14$, $P < .0001$, df = 228, 459; for nonhost vs. nonhost, $F = 230.74$, $P < .0001$, df = 180, 303). The difference between the blue tit and peafowl visual system was more pronounced when comparing pairs of host species (host vs. host) than when comparing nonhosts ($F = 150.34$ and $F = 91.00$, respectively). This means that an animal with a blue tit–like visual system would detect even more color differences between host eggs than would an animal with a visual system like that of the peafowl. The same analyses were performed using a Weber fraction of 0.02 instead of 0.05, and the results remain qualitatively the same. Despite the fact that the values of JNDs are higher for the blue tit visual system, all other analyses shown below had the same qualitative results for both visual systems. Thus, from now on, we will refer only to the analyses employing the blue tit visual system and a Weber fraction of 0.05.

New Hypothesis: Diversity of Egg Color and Pattern among Species

Overall, phylogenetic distance was a good predictor of differences in egg color and in egg pattern between species (table 1; fig. 2). However, phylogenetic distance had a weaker relationship with egg pattern differences between hosts than between nonhosts, as indicated by the significant differences in slope in figure 2. Moreover, in addition to the effect of phylogenetic distance, differences in egg pattern between host species were significantly higher than the differences between nonhost species. This means that, over the same period of evolutionary time, two host species will evolve more differences in egg pattern than two nonhost species. However, this was not true for color (table A1). There are no significant differences in JNDs for comparisons between hosts and nonhosts. All the findings described above remained the same after doing the analyses using 1,000 different phylogenetic trees. Results remained the same both after excluding species with closed nests and after excluding species with immaculate eggs (fig. A4). Furthermore, among hosts, pattern differences were significantly smaller between pairs of hosts that share the same parasite, after controlling for phylogenetic distance ($\beta = -14.55 \pm 3.71$, $P = .00016 \pm .0001$; fig. 3).

Classic Predictions: Variation in Egg Pattern within Species and within Clutches

Eggs of host species were significantly more polymorphic in egg pattern than were those of nonhost species (fig. 4). Specifically, differences in peak energy were significantly higher between individuals of host species (within-species variation), and differences in peak frequency are significantly higher within clutches of host species (within-clutch

Table 1: Mean statistics of egg phenotypic diversity between species

	Differences in egg pattern		JNDs in speckle color		JNDs in background color	
Predictor	β	P value	β	P value	β	P value
Phylogenetic distance	.003 ± .0003	**.038 ± .012**	.03 ± .0005	**.031 ± .02**	.012 ± .001	**.0008 ± .006**
Type of comparison	.90 ± .07	**.0006 ± .0007**	.30 ± .082	.198 ± .123	.121 ± .147	.607 ± .227
Phylogenetic distance × type of comparison	.006 ± .0007	**.013 ± .010**	.007 ± .0008	.098 ± .08	.009 ± .001	.614 ± .231

Note: We report the slope value and the significance for each predictor. Standard deviations were obtained from doing the analyses with 1,000 different phylogenetic hypotheses. JNDs = just noticeable differences. “Type of comparison” refers to whether the distance was calculated between two host species or two nonhost species. Significant P values are in bold type.

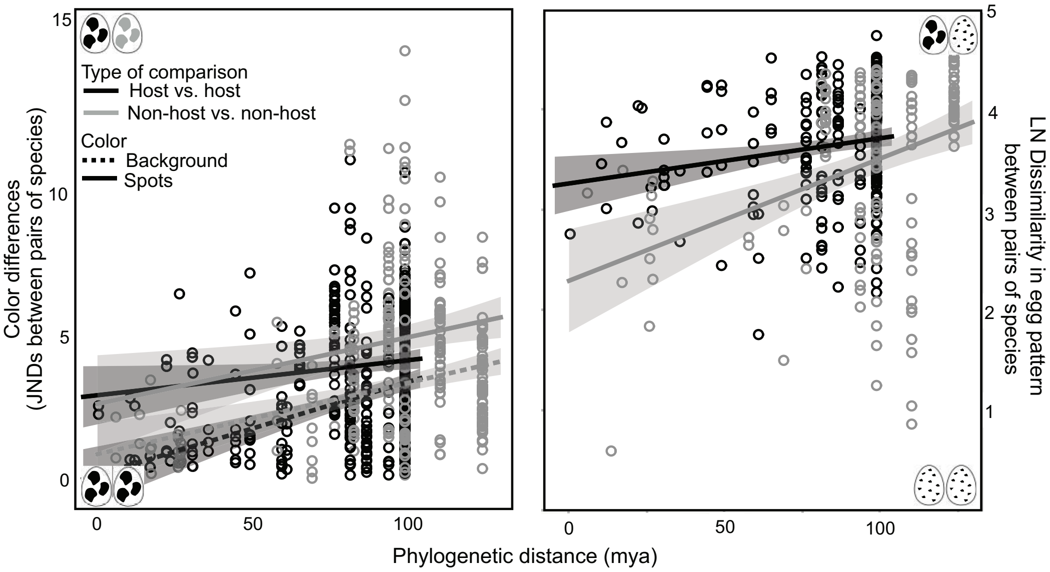


Figure 2: Phylogenetic distance explains differences between species in both egg coloration (*left*) and egg pattern (*right*). In the case of egg pattern, differences are significantly higher for host versus host comparisons (black) than for nonhost versus nonhost comparisons (gray). JND = just noticeable difference; LN = lognormal; mya = million years ago.

variation). Differences remained significant after doing the analyses for 1,000 different phylogenetic trees (pgls, peak frequency ± SD: $\lambda = 0 - 0.762$, $\beta = 28.81 \pm 13.980$, $P = .0461 \pm .0001$; peak energy ± SD: $\lambda = 0 - 0.438$, $\beta = 2.22 \pm 1.057$, $P = .041 \pm 1 \times 10^{-4}$). We found no evidence for differences in color variation or in total energy; hosts were no more polymorphic than nonhosts in egg background color ($\beta = -0.021$, $P = .251$), speckle color ($\beta = -0.075$, $P = .483$), or contrast ($\beta = 3.25$, $P = .45$).

Discussion

In this study, we aimed to explain avian egg diversity among species by testing the role of brood parasitism in the evolution of egg phenotype. By using a data set of Australian passerines that are hosts and nonhosts of different cuckoo species, we demonstrate that, in the same system, brood parasitism is associated with egg phenotypic variation within individuals, within species, and among species. We show for the first time, to our knowledge, that pairs of host species have diverged more in egg pattern than pairs of nonhost species. Additionally, divergence in egg phenotype is smaller in hosts that are exploited by the same parasite than in those exploited by different parasites.

Traditional hypotheses to explain egg phenotypic diversity include thermoregulation and camouflage against predation (Westmoreland et al. 2007; Mayer et al. 2009). However, coevolution with brood parasites may also be responsible for phenotypic differences across species. Our analyses show that host species are likely to evolve more egg pattern differences among them than nonhost species, even if they have evolved independently for the same length of time. This supports a scenario where host species may escape a parasitic egg phenotype through the evolution of different strategies or different adaptive phenotypes, resulting in divergence. Our study also shows that egg pattern evolves differently in hosts and nonhosts. Under a neutral scenario, the expectation is that phenotypic differences between two species should increase in relation to the time since divergence (Martins 1994). This scenario is supported by the results for nonhost species (fig. 2). However, the slope of the relationship between phylogenetic distance and egg pattern dissimilarity is significantly less steep for the host versus host comparison (fig. 2). This suggests that phylogenetic history is less important than selective pressures in shaping the differences in egg pattern between host species. The results do not differ between open nesters and dome nesters (fig. A4), suggesting that possible egg rejection differences between hosts with different nest types are not critical at this macroevolutionary scale. Possible explanations for this are that (i) the effect is sufficiently strong to persist even with the inclusion of a few species with low rates of egg rejection, (ii) even low rates of egg rejection have evolutionary consequences, and (iii) the egg morphology of

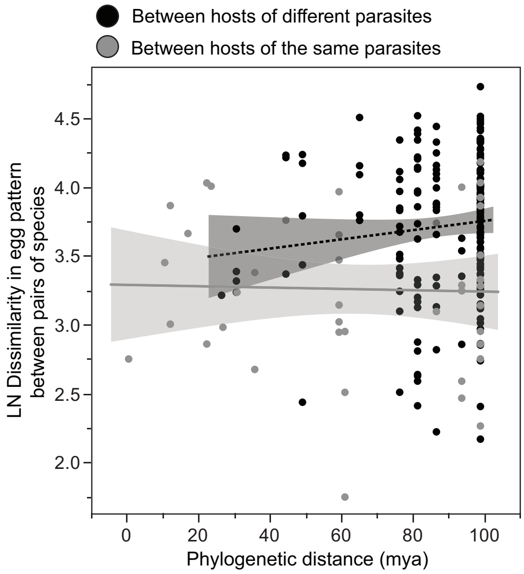


Figure 3: Levels of egg pattern dissimilarity are significantly higher between host species of different parasites (black) than between hosts of the same parasite species (gray). LN = lognormal; mya = million years ago.

dome-nesting hosts may be under selection as a result of selective egg removal by the parasite (Gloag et al. 2014).

Our study shows that brood parasitism is associated with an increase in egg pattern divergence among host species and across different families. Moreover, we show that parasite diversity increases the likelihood of divergence. The egg pattern of hosts that are exploited by the same brood parasite was less diverse than that of hosts exploited by different parasites (fig. 3). This trend was to be expected, given that hosts of the same parasite are often phylogenetically related, so their eggs may be more similar from the outset, and they may be more likely to evolve along similar evolutionary trajectories.

We have shown that the eggs of host species have evolved more phenotypic differences than those of nonhost species, controlling for divergence time. Correspondingly, it is also possible that, in nonhost species, there are additional stabilizing selection sources on egg phenotype that hinder variability, such as camouflage and thermoregulation (Stuart-Fox and Moussalli 2009; Vignieri et al. 2010; Hegna et al. 2013), whereas brood parasitism is the leading selective pressure in hosts.

We found no evidence to suggest that particular egg colors or patterns were associated with host status. Both immaculate and maculated eggs can be found in host and nonhost species. However, we found that host species were more variable in pattern than nonhost species. This supports a well-established hypothesis that a host's egg phenotype will evolve in random directions away from that of the parasite, thereby increasing within-species variation over time (Soler and Møller 1995; Lahti 2005; Spottiswoode and Stevens 2012). High variation in egg phenotype within species has been reported for hosts in comparative analyses done in other systems (Soler and Møller 1995; Kilner 2006). Moreover, eggs of European host species tend to be more polymorphic than those from North America, and this difference has been linked to the low specificity of the host-parasite system in the latter (Stokke et al. 2002). Our results also suggest that variation in the pattern, but not the color of the background or the speckles, is influenced by selection from brood parasitism, since we found no differences in variation between hosts and nonhosts for color traits. Our results correspond with studies of the European cuckoo, which show that pattern is an informative trait that increases likelihood of discrimination (Stoddard and Stevens 2010). Similarly, in passerines such as the village weaver *Ploceus cucullatus* and the bush warbler *Cettia diphone*, the presence of spots and their density are known to influence rejection probability (Higuchi 1998; Lahti and Lahti 2002).

Contrary to theoretical predictions, we found that within-clutch variation was also higher for hosts than nonhosts. Our study is the first comparative analysis to find this significant trend across different species. In theory, within-clutch variation should decrease in hosts, because this should facilitate discrimination between the host's own eggs and foreign eggs (Davies and Brooke 1989*b*; Jackson 1998). In support of this theory, reed warblers are more likely to reject foreign eggs if they lay a more uniform clutch (Stokke et al. 1999; Moskát et al. 2008). However, many empirical (Avilés et al. 2004; Cherry et al. 2007; Landstrom et al. 2010) and comparative analyses (Soler and Møller 1995; Stoddard et al. 2014) fail to support this hypothesis, and Cherry et al. (2007) found that, in a common cuckoo host, the great reed warbler (*Acrocephalus arundinaceus*), rejection of cuckoo eggs improved with increasing within-clutch variation (Cherry et al. 2007). Avilés et al. (2004) also reported that magpie hosts of the great-spotted cuckoo rejected more model eggs if the clutch was highly variable (Avilés et al. 2004). Stoddard et al. (2014) found that eggs could be easily recognizable in species with elevated levels of within-clutch variation if such variation is associated with having distinctive egg signatures (e.g., particular blotches and markings), such as in the brambling (*Fringilla montifringilla*). In Australian hosts, high within-clutch variation may also be related to the evolution of particular pattern signatures; however, this hypothesis remains to be tested.

Although the cause-effect relationship is hard to test, as in any other comparative analyses, three previous studies

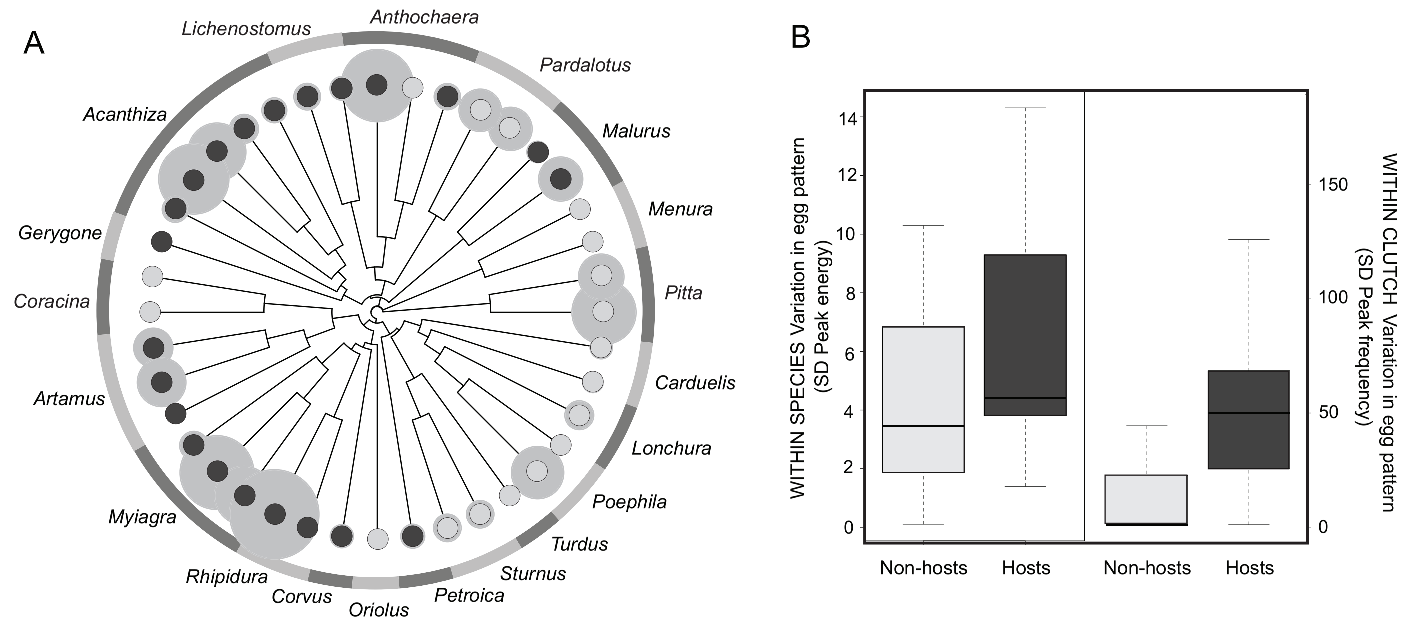


Figure 4: Host species have more variable eggs than nonhost species. *A*, Phylogenetic distribution of host status (host = black; nonhost = light gray) and level of within-species variation (size of gray circle). *B*, Variation in egg pattern is significantly higher in hosts both within the clutch and within species.

on the evolution of polymorphism in hosts strongly suggest that brood parasitism influences egg phenotype and not the other way around. Spottiswoode and Stevens (2012) showed that host species of brood parasites increase their egg variability after only 40 years of parasitism, and Lahti (2005) and Yang et al. (2014) demonstrated that egg variability is reduced when hosts are released from selection by brood parasites (Lahti 2005; Yang et al. 2014). Although we cannot completely reject the hypothesis that parasites selectively target hosts with high levels of variation, such a scenario seems implausible, because the evolution of egg mimicry in brood parasites would be constrained, leading to higher rates of rejection of brood parasite eggs.

Finally, we have shown that brood parasitism is associated with the generation of egg diversity at different taxonomic levels. Currently, there is no information on egg rejection rates for most of the species that we used in this study, but on the basis of our results, we predict high rates of egg rejection as a defense in these species. In fact, egg rejection has a high phylogenetic signal (Medina and Langmore 2015), and the average rejection levels for other Australian species from the same genera are high (77.1% for open nesters and 12.66% for dome nesters; table A1). We would predict that similar systems of coevolution between hosts and highly virulent parasites, such as the European cuckoo, should also exhibit the evolutionary pattern that we report, because rejection rates are high in many European hosts (Langmore et al. 2005). Moreover, studying different components of egg phenotype allowed us to identify pattern as a more important trait than color. By using detailed data on numerous species, we were able to show that brood parasitism has deep implications for egg phenotype and is influential enough to leave traces at a larger evolutionary scale than previously studied.

Acknowledgments

We thank L. Joseph and R. Palmer for access to the Australian National Wildlife Collection egg collection and R. Vega, T. Wallenius, and two anonymous reviewers for very helpful comments on previous versions of the manuscript. I.M. was supported by an Australian National University Vice-Chancellor's travel grant, and N.E.L. was supported by the Australian Research Council.

Literature Cited

Avilés, J. M., and A. Møller. 2003. Meadow pitpit egg appearance in cuckoo sympatric and allopatric populations. Biological Journal of the Linnean Society 79:543–549.

Avilés, J. M., J. J. Soler, M. Soler, and A. P. Møller. 2004. Rejection of parasitic eggs in relation to egg appearance in magpies. Animal Behaviour 67:951–958.

Avilés, J. M., J. R. Vikan, F. Fossøy, A. Antonov, A. Moksnes, E. Røskaft, and B. G. Stokke. 2010. Avian colour perception predicts behavioural responses to experimental brood parasitism in chaffinches. Journal of Evolutionary Biology 23:293–301.

Beruldsen, G. 2003. Australian birds, their nests and eggs. Kenmore Hills, Queensland.

Brooker, M. G., and L. C. Brooker. 1989. Cuckoo hosts in Australia. Australian Zoological Reviews 2:1–67.

Campbell, F. W., and G. Robson. 1968. Application of Fourier analysis to the visibility of gratings. Journal of Physiology 197:551–566.

Caves, E., M. Stevens, E. Iversen, and C. Spottiswoode. 2015. Hosts of avian brood parasites have evolved egg signatures with elevated information content. Proceedings of the Royal Society B: Biological Sciences 282(1810). pii: 20150598.

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

Cuthill, I. 2006. Mechanisms and measurement. Pages 3–40 *in* G. E. Hill and K. J. McGraw, eds. Bird coloration. Harvard University Press, Cambridge, MA.

Davies, N. B. 2000. Cuckoos, cowbirds and other cheats. Poyser, London.

Davies, N. B., and M. de L. Brooke. 1989*a*. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. Journal of Animal Ecology 58:207–224.

———. 1989*b*. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. Journal of Animal Ecology 58:225–236.

del Hoyo, J., A. Elliott, J. Sargata, D. A. Christie, and E. de Juana. 2014. Handbook of the birds of the world alive. Lynx, Barcelona.

de Mársico, M. C., M. G. Gantchoff, and J. Reboreda. 2012. Host-parasite coevolution beyond the nestling stage? mimicry of host fledglings by the specialist screaming cowbird. Proceedings of the Royal Society B: Biological Sciences 279:3401–3408.

Feeney, W. E., M. C. Stoddard, R. M. Kilner, and N. E. Langmore. 2014. "Jack of all trades" egg mimicry in the brood parasitic Horsfield's bronze-cuckoo. Behavioral Ecology 25:1365–1373.

Feeney, W. E., J. A. Welbergen, and N. E. Langmore. 2012. The frontline of avian brood parasite-host coevolution. Animal Behaviour 84: 3–12.

Gloag, R., L. Keller, and N. E. Langmore. 2014. Cryptic cuckoo eggs hide from competing cuckoos. Proceedings of the Royal Society B: Biological Sciences 281:1–7.

Godfrey, D. J., J. N. Lythgoe, and D. A. Rumball. 1987. Zebra stripes and tiger stripes: the spatial frequency distribution of the pattern compared to that of the background is significant in display and crypsis. Biological Journal of the Linnean Society 32:427–433.

Grim, T. 2007. Experimental evidence for chick discrimination without recognition in a brood parasite host. Proceedings of the Royal Society B: Biological Sciences 274:373–381.

Hart, N. S. 2001. The visual evology of avian photoreceptors. Progress in Retinal and Eye Research 20:675–703.

Hart, N. S., and D. M. Hunt. 2007. Avian visual pigments: characteristics, spectral tuning and evolution. American Naturalist 169(suppl.): S7–S26.

Hegna, R. H., O. Nokelainen, J. R. Hegna, and J. Mappes. 2013. To quiver or to shiver: increased melanization benefits thermoregulation, but reduces warning signal efficacy in the wood tiger moth. Proceedings of the Royal Society B: Biological Sciences 280:1–9.

Higuchi, H. 1998. Host use and egg color of Japanese cuckoos. Pages 80–93 *in* S. I. Rothstein and S. K. Robinson, eds. Parasitic birds and their hosts: studies in coevolution. Oxford University Press, New York.

Jackson, W. M. 1998. Egg discrimination and egg-color variability in the Northern Masked Weaver. Pages 407–416 *in* S. I. Rothstein and S. K. Robinson, eds. Parasitic birds and their hosts: studies in coevolution. Oxford University Press, Oxford.

Jetz, W., G. Thomas, A. Hartmann, and O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444–448.

Kilner, R. M. 2005. The evolution of virulence in brood parasites. Ornithological Science 4:55–64.

———. 2006. The evolution of egg color and patterning in birds. Biological Reviews 81:383–406.

Lahti, D. C. 2005. Evolution of bird eggs in the absence of cuckoo parasitism. Proceedings of the National Academy of Sciences of the USA 102:18057–18062.

Lahti, D. C., and A. R. Lahti. 2002. How precise is egg discrimination in weaverbirds? Animal Behaviour 62:1135–1142.

Landstrom, M. T., R. Heinsohn, and N. E. Langmore. 2010. Clutch variation and egg rejection in three hosts of the pallid cuckoo *Cuculus pallidus*. Behaviour 147:19–36.

Langmore, N., and R. Kilner. 2009. Why do Horsfield's bronze-cuckoo *Chalcites basalis* eggs mimic those of their hosts? Behavioral Ecology and Sociobiology 63:1127–1131.

Langmore, N. E., W. E. Feeney, J. Crowe-Riddell, H. Luan, K. M. Louwrens, and A. Cockburn. 2012. Learned recognition of brood parasitic cuckoos in the superb fairy-wren *Malurus cyaneus*. Behavioral Ecology 23:798–805.

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

Langmore, N. E., R. M. Kilner, S. H. M. Butchart, G. Maurer, N. B. Davies, A. Cockburn, N. A. Macgregor, et al. 2005. The evolution of egg rejection by cuckoo hosts in Australia and Europe. Behavioral Ecology 6:686–692.

Langmore, N. E., and C. N. Spottiswoode. 2012. Visual trickery in avian brood parasites. Pages 95–118 *in* D. Highes, J. Brodeur, and F. Thomas, eds. Host manipulation by parasites. Oxford University Press, Oxford.

Legendre, P., and M. J. Fortin. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Molecular Ecology Resources 10:813–844.

Martins, E. 1994. Estimating the rate of phenotypic evolution from comparative data. American Naturalist 144:193–209.

Mayer, P. M., N. G. Smith, R. G. Ford, D. C. Watterson, M. D. McCutchen, and M. R. Ryan. 2009. Nest construction by a ground-nesting bird represents a potential trade-off between egg crypticity and thermoregulation. Oecologia 159:893–901.

Medina, I., J. Troscianko, M. Stevens, and N. E. Langmore. 2016. Data from: Brood parasitism is linked to egg pattern diversity within and among species of Australian passerines. American Naturalist, Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.s31sp.

Medina, I. M., and N. E. Langmore. 2015. The costs of avian brood parasitism explain variation in egg rejection behaviour in hosts. Biology Letters 11:1–5.

Moksnes, A., E. Røskaft, and A. T. Braa. 1991. Rejection behavior by common cuckoo hosts towards artificial brood parasite eggs. Auk 108:348–354.

Moskát, C., J. M. Avilés, M. Ban, and A. Zölei. 2008. Experimental support for the use of egg uniformity in parasite egg discrimination by cuckoo hosts. Behavioral Ecology and Sociobiology 62:1885–1890.

Ödeen, A., S. Pruett-Jones, A. Driskell, J. K. Armenta, and O. Håstad. 2012. Multiple shifts between violet and ultraviolet vision in a family of passerine birds with associated changes in plumage coloration. Proceedings of the Royal Society B: Biological Sciences 279: 1269–1276.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2012. Caper: comparative analyses of phylogenetics and evolution in R. R package version 0.5.

Osorio, D., and M. Voroyev. 2005. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. Proceedings of the Royal Society B: Biological Sciences 272:1745–1752.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:11.

Payne, R. B., and L. L. Payne. 1998. Parasitic birds and their hosts. Studies in Coevolution. Oxford University Press, Oxford.

Rasband, W. S. 2014. Image J. National Institutes of Health. http://imagej.nih.gov/ij/.

Rothstein, S. I. 1975. Experimental and teleonomic investigation of avian brood parasitism. Condor 77:250–271.

Sato, N. J., K. Tokue, R. A. Noske, O. K. Mikami, and K. Ueda. 2010. Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. Biology Letters 6:67–69.

Siddiqi, A., T. Cronin, E. R. Loew, M. Vorobyev, and K. Summers. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. Journal of Experimental Biology 207:2471–2485.

Soler, J. J., and A. Møller. 1995. A comparative analysis of the evolution of variation in appearance of eggs of European passerines in relation to brood parasitism. Behavioural Ecology 7:89–94.

Soler, M. 2014. Long-term coevolution between avian brood parasites and their hosts. Biological Reviews 89:688–704.

Spottiswoode, C. N., and M. Stevens. 2010. Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs. Proceedings of the National Academy of Sciences of the USA 107:8672–8676.

———. 2011. How to evade a coevolving brood parasite: egg discrimination versus egg variability as host defences. Proceedings of the Royal Society B: Biological Sciences 278:3566–3573.

———. 2012. Host-parasite arms races and rapid changes in bird egg appearance. American Naturalist 179:633–648.

Starling, M., R. Heinsohn, A. Cockburn, and N. E. Langmore. 2006. Cryptic gentes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. Proceedings of the Royal Society B: Biological Sciences 273:1929–1934.

Stevens, M., A. Parraga, I. Cuthill, C. Partridge, and T. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90:211–237.

Stevens, M., J. Troscianko, and C. N. Spottiswoode. 2013. Repeated targeting of the same hosts by a brood parasite compomises host egg rejection. Nature Communications 4:3–7.

Stoddard, M. C., R. M. Kilner, and C. Town. 2014. Pattern recognition algorithm reveals how birds evolve individual egg pattern signatures. Nature Communications 5:1–5.

Stoddard, M. C., and M. Stevens. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. Proceedings of the Royal Society B: Biological Sciences 277:1387–1393.

———. 2011. Avian vision and the evolution of egg color mimicry in the common cuckoo. Evolution 65:2004–2013.

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

Stokke, B. G., A. Moksnes, E. Røskaft, S. Rudolfsen, and M. Honza. 1999. Rejection of artificial cuckoo (*Cuculus canorus*) eggs in relation to variation in egg appearance among reed warblers (*Acrocephalus scirpaceus*). Proceedings of the Royal Society B: Biological Sciences 266:1483–1488.

Stokke, B. G., G. Rudolfser, A. Moksnes, and E. Røskaft. 2004. Rejection of conspecific eggs in chaffinches: the effect of age and clutch characteristics. Ethology 110:459–470.

Stuart-Fox, D., and A. Moussalli. 2009. Camouflage, communication and thermoregulation: lessons from color changing organisms. Philosophical Transactions of the Royal Society B: Biological Sciences 364:463–470.

Swynnerton, C. F. M. 1918. Rejections by birds of eggs unlike their own: with remarks on some of the cuckoo problems. Ibis 60:127–154.

Troscianko, J. 2014. A simple tool for calculating egg shape, volume and surface area from digital images. Ibis 156:874–878.

Underwood, T. J., and S. G. Sealy. 2002. Adaptive significance of egg coloration. Pages 280–289 *in* D. C. Deeming, ed. Avian incubation: behaviour, environment and evolution. Oxford University Press, Oxford.

Victoria, J. K. 1972. Clutch characteristics and egg discriminative ability of the African village weaver bird *Ploceus cucullatus*. Ibis 114: 367–376.

Vignieri, S., J. Larson, and H. Hoekstra. 2010. The selective advantage of crypsis in mice. Evolution 64:2153–2158.

Vorobyev, M., and D. Osorio. 1998. Receptor noise as a determinant of color thresholds. Proceedings of the Royal Society B: Biological Sciences 265:351–358.

Welbergen, J. A., and N. B. Davies. 2009. Strategic variation in mobbing as a front line of defense against brood parasitism. Current Biology 19:235–240.

Westmoreland, D., M. Schmitz, and K. E. Burns. 2007. Egg color as an adaptation for thermoregulation. Journal of Field Ornithology 78:176–183.

Yang, C., Y. Liu, L. Zeng, and W. Liang. 2014. Egg color variation, but not egg rejection behavior, changes in a cuckoo host breeding in the absence of brood parasitism. Ecology and Evolution 4:2239–2246.

Associate Editor: Edmund D. Brodie III
Editor: Yannis Michalakis

Chapter 8

Coevolution is linked with phenotypic diversification but not speciation in avian brood parasites.

Published in *Proceedings of the Royal Society*.

PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Medina I, Langmore NE. 2015 Coevolution is linked with phenotypic diversification but not speciation in avian brood parasites. *Proc. R. Soc. B* **282**: 20152056.
http://dx.doi.org/10.1098/rspb.2015.2056

Received: 26 August 2015
Accepted: 19 November 2015

Subject Areas:
behaviour, ecology, evolution

Keywords:
coevolution, diversification, phenotypic rates, brood parasitism, speciation

Author for correspondence:
Iliana Medina
e-mail: iliana.medina@anu.edu.au

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.2056 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY
PUBLISHING

Coevolution is linked with phenotypic diversification but not speciation in avian brood parasites

Iliana Medina and Naomi E. Langmore

Department of Ecology, Evolution and Genetics, Australian National University, Canberra 0200, Australian Capital Territory 2601, Australia

Coevolution is often invoked as an engine of biological diversity. Avian brood parasites and their hosts provide one of the best-known examples of coevolution. Brood parasites lay their eggs in the nests of other species, selecting for host defences and reciprocal counteradaptations in parasites. In theory, this arms race should promote increased rates of speciation and phenotypic evolution. Here, we use recently developed methods to test whether the three largest avian brood parasitic lineages show changes in rates of phenotypic diversity and speciation relative to non-parasitic lineages. Our results challenge the accepted paradigm, and show that there is little consistent evidence that lineages of brood parasites have higher speciation or extinction rates than non-parasitic species. However, we provide the first evidence that the evolution of brood parasitic behaviour may affect rates of evolution in morphological traits associated with parasitism. Specifically, egg size and the colour and pattern of plumage have evolved up to nine times faster in parasitic than in non-parasitic cuckoos. Moreover, cuckoo clades of parasitic species that are sympatric (and share similar host genera) exhibit higher rates of phenotypic evolution. This supports the idea that competition for hosts may be linked to the high phenotypic diversity found in parasitic cuckoos.

1. Introduction

Coevolution has long been thought to be instrumental in the evolution of phenotypic and species diversity. In their highly influential review, Ehrlich & Raven [1, p. 606] stated that 'The importance of reciprocal selective responses between ecologically closely linked organisms has been vastly underrated in considerations of the origins of organic diversity'. However, not all coevolutionary interactions are expected to lead to diversification [2]. For instance, it has been suggested that mutualistic interactions can actually hinder diversity, whereas host–parasite interactions, specifically brood parasitism, should promote diversification [2].

Here, we capitalize on new techniques for quantifying rates of speciation and phenotypic evolution [3] to test this prediction using one of the best-known coevolutionary systems, the interactions between avian brood parasites and their hosts. Avian interspecific brood parasites lay their eggs in the nests of other species, which eventually take care of the parasitic chick [4]. This interaction is so costly to the hosts that it has led to the evolution of defences such as mobbing of brood parasites [5] and discrimination and rejection of parasitic eggs and chicks [6,7]. Defences in hosts have, in turn, selected for counteradaptations in brood parasites, and many brood parasite species have evolved eggs or chicks that mimic the morphology of their hosts [8,9]. These tightly coupled interactions give rise to a rapidly evolving arms race, where changes in host and parasite phenotypes can be detected in just 40 years [10].

Brood parasitism has at least seven independent origins in the avian phylogeny [4,11]. However, large lineages of avian brood parasites (more than five spp.), suitable for this type of analysis, have evolved only three times, in the subfamily Cuculinae (Old World cuckoos, 52 spp.), and in the families Indicatoridae (honeyguides, 17 spp.) and Viduidae (Vidua finches, whydahs and the cuckoo finch, 20 spp.) [12,13]. These three lineages differ in their impact on hosts. Most of the cuckoos and the honeyguides are highly virulent, because their chicks kill or outcompete the progeny of the host [14]. Vidua finches and whydahs, on the other hand, impose lower costs on their hosts, because their young can be raised alongside the host chicks [15]. Differences in virulence between parasitic lineages may have repercussions in the coevolutionary interactions with their hosts, as selection for defences against parasitism is likely to be stronger in hosts of the more virulent species, leading to more rapid evolution of defences in hosts and counteradaptations in parasites [15].

There is some evidence to suggest that coevolution between avian brood parasites and their hosts can lead to increased rates of divergence in brood parasites. Rapid genetic divergence may occur when different populations of a generalist species become more specialized on particular host species, or when they exploit, and specialize on, novel host species. For example, brood parasitic Vidua finches, *Vidua* spp., underwent sympatric speciation as they annexed novel host species [15]. Moreover, specialization on particular host species has led to genetic divergence within some cuckoo and honeyguide species into distinct host-specific races [16–18], and Krüger *et al.* [19] demonstrated that species of parasitic cuckoos had more subspecies than species of non-parasitic cuckoos. Since the appearance of these studies, novel phylogenies [20] and phylogenetic methods [3,21] have been developed.

Coevolution with hosts may also affect rates of phenotypic evolution, at least in traits that facilitate parasitism. The evolution of parasitic behaviour in cuckoos is associated with a reduction in egg size and possibly body size [22], perhaps because hosts of highly virulent brood parasites are usually much smaller than their parasite. Smaller body size is likely to be particularly adaptive in the *Chrysococcyx* and *Chalcites* cuckoos, because these genera parasitize nests with small entrances [22]. Adaptations of plumage colour and pattern have also been suggested to facilitate parasitism in cuckoos. Many cuckoos exhibit a combination of yellow legs, yellow eye ring, barred chest plumage and cryptic phenotype (e.g. no crests or conspicuous colours), which are believed to mimic the morphology of hawks, as a means of intimidating hosts [23]. Selection for mimicry of sympatric hawk species has even been shown to give rise to plumage polymorphisms within cuckoo species [24]. If the traits mentioned above are host-specific and the adaptive value varies depending on which host is exploited, then we may expect increased phenotypic diversity and a change in the rates of evolution of these specific traits in brood parasitic lineages.

In this study, we use the three largest clades of avian brood parasites (cuckoos, honeyguides and Vidua finches) to explore whether these taxa show faster rates of speciation and phenotypic evolution compared with closely related clades. Because host use can be related to diversity in coevolutionary interactions [25,26], we also test whether different measures of host diversity can explain the patterns observed. We calculate the *breadth* of host niche within the three lineages of parasites, which is a measure of how phylogenetically distant their hosts are from each other in the avian phylogeny. Because parasitic cuckoos are the largest and best-studied clade (e.g. with enough reliable host information), we also calculate an index of host *overlap* between parasitic species within seven cuckoo clades (mostly genera). This index shows whether species within a clade share the same host genera (high overlap) or parasitize different host genera (low overlap). This measure is related to the geographical overlap between species, which we also calculate.

We predict that (i) parasitic lineages with wider host breadth will have higher rates of both speciation and phenotypic diversity, given their wide host variety and (ii) host use and geographical distribution may explain the variation in rates of evolution between clades of cuckoos. Namely, in sympatric clades, where parasitic species tend to share the same host genera, parasites will be more likely to present similar adaptations and thus phenotype will evolve more slowly, in contrast to clades in which each species parasitizes different genera (low host overlap).

2. Methods

(a) Species used and traits measured

To test whether rates of evolution are more rapid in parasitic lineages than in non-parasitic lineages, we sampled species from three parasitic lineages and species from their most closely related non-parasitic clade. For parasitic cuckoos (52 spp.), we used the non-parasitic cuckoos (91 spp.) for comparison, for honeyguides (17 spp.), we used the woodpeckers (Picidae, 222 spp.) for comparison and for the Vidua finches (20 spp.), we used the Estrildid finches (140 spp.) and the African weavers (116 spp.). We used two lineages for comparison in Vidua finches, because the Estrildid finches are the most closely related group, but are also the main hosts of this family, and this could make the comparison problematic if these also show changes in rates of evolution in response to brood parasitism. For diversification rates analyses, we sampled 100% of the targeted parasitic species and from 98% to 100% of the sister species. For the phenotypic analyses, samples varied depending on available information, for colour approximately 95%, for size approximately 90% and for egg size from 48% to 82% of sister species. In this last case, we ran complementary analyses to assess the effect of incomplete sampling (electronic supplementary material, Material and methods). To assess the effect of phylogenetic uncertainty, we sampled 10 random possible phylogenetic trees from a pseudo-posterior distribution from www.birdtree.org [20] for each of the lineages where parasitism evolved independently (Cuculidae, Indicatoridae and Viduidae). We used 10 trees for each analysis based on the fact that previous studies have used three or four phylogenies [27]. Results were highly consistent across trees.

We investigated the evolution of three different morphological traits (plumage, egg size and body size). For the family Cuculidae, we obtained information on egg size and body size from published studies [24,28,29]. We also generated an index of hawk-like plumage by combining variables previously published in two different studies, specifically, presence of barring in the breast plumage, yellow eye ring, yellow legs, crest presence and presence of cryptic colours [24,28]. Additionally, we used the plates available in the Handbook of Birds of the World online [30] to quantify overall plumage coloration, using spectral measurements of museum specimens to validate our method (electronic supplementary material, Material and methods). For the analyses on honeyguides and Vidua finches, we collected information on body size from the *Handbook of Birds of the World online* [30], and information on egg size from the *Handbook of Oology* [in German] [31] and *A Guide to the Nests and Eggs of Southern African Birds* [32]. To quantify plumage differences in Vidua finches and honeyguides, we repeated the procedure using plates from the *Handbook of Birds of the World* [30] as described above.

(b) Modelling rates of evolution

To explore rates of speciation and rates of trait evolution, we used the software BAMM [3], which uses reversible jump Markov chain Monte Carlo to automatically detect and quantify shifts in evolutionary rates. We chose this over other methods because it does not require any *a priori* information on whether the species are brood parasites or not. Thus, if brood parasitism were associated with changes in rates in these lineages, then we would expect a shift in rates in the same node where brood parasitism originated or in a subsequent node; conversely, shifts detected in other nodes, or no shifts would suggest that there is no association between changes in rates and the evolution of brood parasitism, following the rationale of Weber & Agrawal [27]. To test whether speciation rates were higher in the three parasitic lineages compared with their closely related clades, we ran 30 analyses (10 per lineage) using the 'diversification' module. This module uses only information on branch lengths and the tree topology to automatically detect changes in rates of speciation and extinction, based on an explicit Bayes factor criterion. For the analyses on plumage, egg size and body size, we used the 'traits' module, and provided trait information for the tips in the phylogeny (more details in electronic supplementary material, Material and methods), and we ran each analysis on 10 different phylogenetic trees. To confirm our results on speciation and extinction rates, we used the R package diversitree [21] and the binary state speciation and extinction model (BiSSE) of Madison *et al.* [33]. To confirm our results on the phenotypic rates, we used the R package phytools (electronic supplementary material, Material and methods). We also used a phylogenetic generalized-least-squares (PGLS) regression using the caper package of R statistics [34] to calculate the correlation in rates of evolution between traits where shifts were detected; for this, we used the values of traits with natural logarithm transformation, following Rabosky *et al.* [35]. In the results, we show an example of an output from one of the 10 trees employed.

(c) Host niche breadth calculation

To estimate the average breadth of host use for each lineage of brood parasites, we used information from host species in southern Africa [36]. By using host species from just one region, and a similar number of parasitic species (cuckoos = 9 spp., Vidua finches = 9 spp. and honeyguides = 6 spp.), we avoid the need to make corrections for geographical distribution of the lineage, because parasites distributed in different continents would probably have access to a different number of host species. With this information, we calculated the average phylogenetic distance between host species for each parasitic lineage, which is a measure of the breadth of distribution of hosts in the phylogeny (electronic supplementary material, Material and methods).

(d) Host niche overlap and geographical overlap in cuckoos

To further explore whether host use could explain rate differences within parasitic cuckoos, we calculated an overlap index in use of host genera and a geographical overlap index. To do this, we divided the parasitic cuckoos into seven monophyletic groups (mostly genera; electronic supplementary material, figure S1), and calculated the degree of host overlap between species within each of the seven clades. The host overlap index tells us on average which proportion of the host genera is shared by different parasites within a clade. Higher overlap means that parasitic species tend to share more host genera. To calculate geographical overlap between each pair of species, we downloaded shape files for each species from www.birdlife.org, then we used the *gIntersection* function in the R package rgeos (electronic supplementary material, Material and methods). It is important to mention that both geographical ranges and host status are dynamic, and the current data are probably different from the historic distributions and hosts, which may obscure and make more difficult the detection of any pattern.

3. Results

(a) Rates of speciation and extinction

Our results from the BAMM software on speciation and extinction rates showed no changes in rates of diversification for any of the three parasitic lineages studied (table 1 and electronic supplementary material, figure S2). However, a significant signal was found by the second method we employed, the BiSSE analysis. In this, we compared the likelihood of models where parasitic lineages had either the same or different diversification rates compared with their sister non-parasitic lineage. We found that the most likely model presented parasites from the Viduidae family with higher rates of speciation and extinction (table 1). There was no evidence for an increase or decrease in speciation or extinction rates in the other two lineages (table 1).

(b) Rates of phenotypic evolution

For the sake of simplicity, from here on, we will refer to 'parasitic cuckoos' as the monophyletic clade of highly virulent cuckoos (Cuculinae), excluding *Clamator, Tapera* and *Dromococcyx*, because we found no differences in any of the analyses between these genera and the non-parasitic cuckoos ($p > 0.05$, see §5). Our analyses show significant differences in the three morphological traits between the parasitic and non-parasitic lineages (figure 1 and electronic supplementary material, table S1). The three parasitic lineages have different plumage coloration, and honeyguides and cuckoos have smaller body size than closely related species (woodpeckers and non-parasitic cuckoos, respectively). These statistical differences are not phylogenetically controlled, because brood parasites are monophyletic in the different lineages studied.

(c) Plumage

Parasitic cuckoos (Cuculinae) showed higher rates of plumage evolution than non-parasitic cuckoos (mean rate parasitic cuckoos = 0.048 ± 0.009, mean rate non-parasitic cuckoos = 0.014 ± 0.002; figure 2*a*). The BAMM analysis detected changes in rates of evolution in the node where brood parasitism evolved in the 10 trees sampled, strongly suggesting that the increase in rate is linked to brood parasitism. Our model analysis supported this, as the model with independent rates for each state was significantly better than the model with one overall rate (mean $\Delta\text{AICc} = 35.5 \pm 4.94$, mean rate parasites = 91.98, mean rate non-parasites = 74.72, $p < 0.0001$). Body size also presented consistently higher rates of evolution in the brood parasitic clade (mean = 0.026) compared with the non-parasitic clade (mean = 0.020), although shifts were not detected.

Plumage coloration in honeyguides shows the opposite pattern, with a slower rate of plumage evolution than their sister clade, the woodpeckers (mean honeyguides = 0.20 ± 0.30, mean woodpeckers = 1.001 ± 0.36, figure 2*c*). Shifts at the base of the honeyguides were detected in nine out of 10 trees. The model analysis confirmed these results (mean $\Delta\text{AICc} = 18.13 \pm 9.87$, mean rate parasites = 15.9, mean rate non-parasites = 253.6, $p < 0.0001$, $\text{ER} > 1000$).

Table 1. Estimation of speciation and extinction rates (average) for each of the three lineages using the BAMM software and using BiSSE models with different and equal rates of speciation (λ) and extinction (μ) for parasites and non-parasites. The Akaike Information Criteria corrected by sample size (AICc) from the constrained BiSSE models was compared with the AICc from the full model (e.g. no constraints) using a Chi-square test and evidence ratios (ERs) and these values are presented in front of each constrained model. ER denotes how many times the full model is better than the constrained one. Only in the case of the Vidua finches is the AICc is significantly better for the full model, and there is a trend of higher extinction and speciation in the parasitic lineage (shown in italics).

	speciation rates λ		extinction rates μ				
lineage and model	not parasitic	parasitic	not parasitic	parasitic	AICc	p(>Chi) (full versus other models)	evidence ratio (full versus other models)
Cuculidae							
BAMM estimation	0.047	0.047	0.006	0.006			
BiSSE full model	0.0446	0.05	0.0106	0	826.68		
BiSSE equal μ	0.0446	0.05	0	0	825.54	0.979	0.565
BiSSE equal λ	0.0466	0.0466	0.0096	0	825.62	0.435	0.588
Indicatoridae							
BAMM estimation	0.21	0.2	0.074	0.068			
BiSSE full model	0.182	0.10325	0.0422	0.015	577.545		
BiSSE equal μ	0.174	0.104	0.115	0.115	573.516	0.595	0.133
BiSSE equal λ	0.1597	0.1597	0.0115	0.0877	577.9075	0.227	0.83
Viduidae							
BAMM estimation	0.211	0.249	0.046	0.073			
BiSSE full model	*0.195*	*0.485*	*0*	*0.458*	*1203.8*		
BiSSE equal μ	0.189	0.636	0	0	1210.3	0.024	33.11
BiSSE equal λ	0.196	0.196	0	0.142	1214	0.085	244.69

(d) Egg size

Our results suggest that there is a higher rate of evolution in egg size in parasitic cuckoos (mean rate parasitic cuckoos $= 0.045 \pm 0.006$, mean rate non-parasitic cuckoos $= 0.005 \pm 0.001$, figure 2*b*). In all the trees sampled, the best shift configuration showed a shift in the node where brood parasitism evolved. The model analysis supported this pattern (mean $\Delta\mathrm{AICc} = 18.8 \pm 3.34$, mean rate parasites $=$ 45.02, mean rate non-parasites $= 20.35$, $p < 0.0001$, $\mathrm{ER} > 1000$). Rates of evolution in egg size and plumage were correlated in parasitic cuckoos (ln values, PGLS $\beta = 0.044$, $p = 0.0003$). There were no shifts detected for egg size in honeyguides or Vidua finches.

(e) Body size

We found no evidence for changes in rates of evolution in body size in any of the three lineages.

(f) Host breadth analysis

Honeyguides and parasitic cuckoos in Africa had a wider host breadth than Vidua finches (figure 3*a*). Honeyguides had the broadest set of hosts (mean phylogenetic distance between hosts = 131.65 Ma, max = 173.61 Ma). Honeyguides not only parasitize passerines (order Passeriformes), but also kingfishers and bee-eaters (order Coraciformes) and woodpeckers (order Piciformes). Parasitic cuckoos in Africa parasitize mainly passerines but occasionally mousebirds (order Coliiformes; mean phylogenetic distance between hosts = 94.87 Ma, max = 167.048). Members of the family Viduidae showed the lowest average phylogenetic distance between hosts; they parasitize passerines exclusively, and the vast majority belong to the family Estrildidae (mean phylogenetic distance between hosts = 53.54 Ma, max = 89.16 Ma).

(g) Host overlap and geographical overlap analyses within cuckoos

Host overlap was related to geographical overlap ($\beta = 0.25$, $p = 0.0001$, figure 3*b*). The clades with the highest degree of overlap in host genera and geographical distribution were *Chrysococcyx* (overlap host = 0.232, geographical overlap = 0.46), which exploits species from the genera *Nectarinia* and *Ploceus*; and *Chalcites* (overlap host = 0.301, geographical overlap = 0.53), which mainly parasitizes species from the genera *Malurus*, *Acanthiza* and *Gerygone*.

Contrary to our prediction, there was a significant positive correlation between both host overlap and

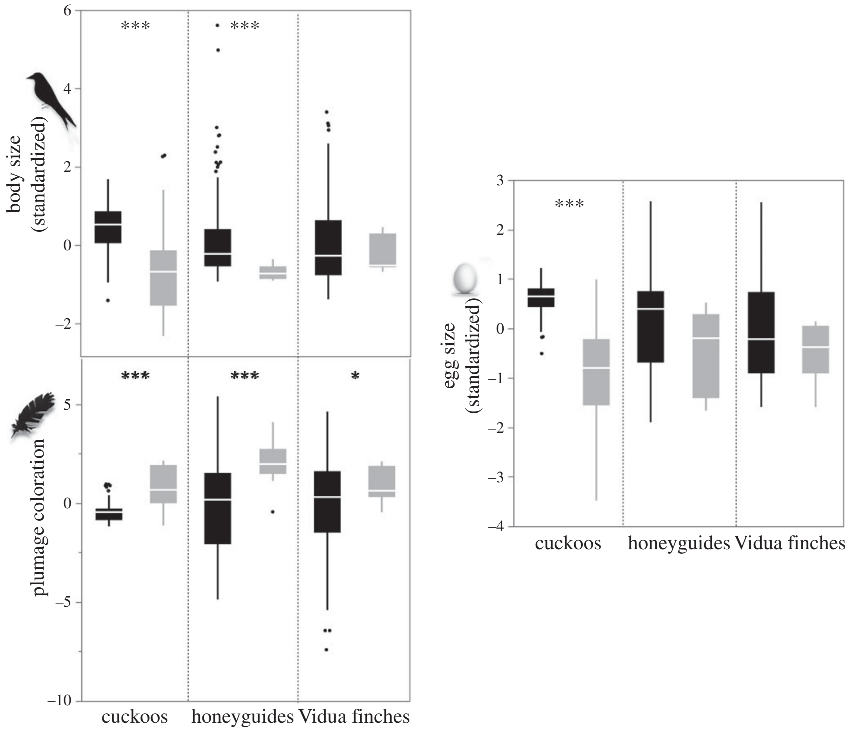


Figure 1. Boxplots of average (±25% of the data) morphological traits measured in non-parasitic (black) and parasitic species (grey) for the three lineages studied (***$p < 0.001$ and *$p < 0.05$). Parasitic lineages tend to have a smaller body size, smaller egg size and different plumage compared with non-parasitic lineages.

geographical overlap with the average rate of plumage evolution (host overlap, $\beta = 1.45$, $p = 0.007$, geographical overlap, $\beta = 34.035$, $p < 0.0001$, figure 3*b*), and a similar pattern for egg size (host overlap $\beta = 0.004$, $p = 0.08$, geographical overlap, $\beta = 3.54$, $p = 0.0019$, figure 3*b*). This association remained true when using host overlap index values of each species, instead of using a value per clade (phylogenetic regression, plumage $\beta = 0.226$, $p = 0.041$, egg size $\beta = 8.18$, $p = 0.011$, electronic supplementary material, figure S3).

4. Discussion

Avian brood parasitism is one of the best examples of coevolution in the animal kingdom, and has been shown to lead to adaptations in both hosts and parasites [4,37]. In theory, coevolution of brood parasites and hosts should lead to increased rates of diversification and increased phenotypic diversity [2,19]. We used two recently developed methods and phylogenies to test these hypotheses explicitly. We found no evidence that honeyguides and parasitic cuckoos have increased speciation or extinction rates; however, in one of the methods employed, we found higher speciation rates for Vidua finches, corroborating the findings of Sorenson *et al.* [15]. Interestingly, both methods employed estimated higher extinction rates for Vidua finches compared with their closest relatives. The lack of higher diversification rates in parasitic cuckoos appears to contrast with the finding of Krüger *et al.* [19] that subspecies richness and cladogenesis was over twice as high in parasitic cuckoos as in parental cuckoos. Our findings highlight the importance of using novel methods and phylogenies to re-evaluate previous findings, and further suggest that the number of subspecies is not necessarily correlated with the actual rates of speciation of a lineage. Although coevolution between brood parasites and their hosts may select for genetic divergence of brood parasites into female host races [38,39], or subspecies [19], this does not necessarily lead to accelerated rates of speciation. Speciation of host races may be constrained by incomplete assortative mating, as has been proposed for the common cuckoo [39] and the greater honeyguide [18].

Regarding trait evolution, we found that the rates of several traits have changed in those nodes where brood parasitic lineages evolved. In parasitic cuckoos (Cuculinae), rates of evolution of coloration were three times faster than in non-parasitic cuckoos. This increase was found for the components associated with hawk-mimicry (e.g. barred-chest, yellow eyes and legs, etc.), but not with other components of colour. This finding supports the hypothesis that hawk-like coloration is an adaptation of parasitic cuckoos [23], and it also suggests that there is high diversity in the specific morphology of this plumage. For instance, the little bronze-cuckoo (*Chalcites minutillus*) sports a barred chest, but this trait has been lost in its close relative, the black-eared cuckoo (*Chalcites osculans*). Moreover, species from the genus *Surniculus* resemble black drongos instead of hawks, which may also be a case of mimicry [4]. Therefore, it is possible that there is diversifying selection on cuckoos to mimic different models, thereby increasing selection for divergence in traits such as barring and colour conspicuousness [40].

The opposite pattern was found for honeyguides, which showed a strong deceleration in rates of plumage evolution.

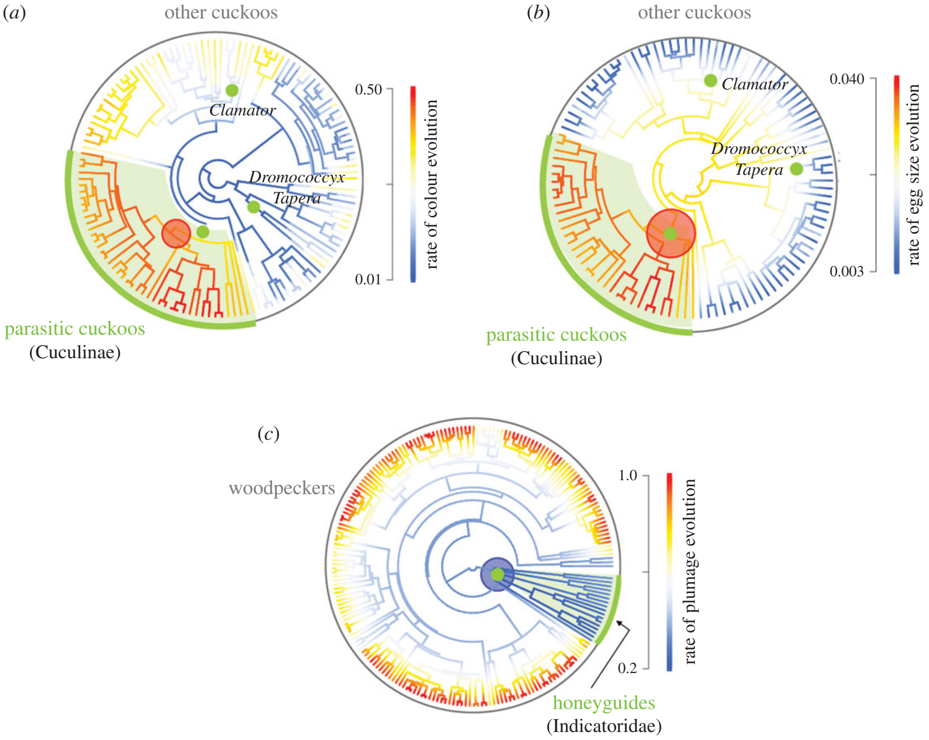


Figure 2. Phylorate plots of BAMM results of rates of phenotypic evolution. Panels (*a*,*b*) show increase in rates of evolution in parasitic cuckoos in coloration and egg size, respectively, and (*c*) shows decrease in rate of plumage evolution in honeyguides. Blue and red circles indicate the position of the most frequent shift in evolutionary rates; a blue circle indicates a shift in rate towards deceleration and red circle indicates a shift towards acceleration. Size of the circle is proportional to the frequency (from 0 to 1) with which the shift was observed. Green circles indicate the basal node of parasitic lineages, and green shading indicates the parasitic species for each dataset. Note that in these three cases the shift in rates occurs at the base of the parasitic lineages, suggesting strong correlation between a change in rates and the evolution of brood parasitism.

Honeyguide species diverged about 10 Ma, allowing ample time for the evolution of plumage divergence, but our results show that plumage varies little between species even though they parasitize hosts from different orders. Although our colour analysis was not performed using spectral measures, we are confident of this pattern of reduced plumage diversity because, if anything, our method tends to overestimate differences between species (electronic supplementary material, Material and methods). Moreover, the pattern we detected is very evident; all honeyguides have dull, pale green or grey plumage, which may be cryptic, and there is no sexual colour dimorphism in most of the species. It would be interesting to know whether dull plumage offers benefits to parasitic females when monitoring or parasitizing host nests, such that plumage divergence is constrained by selection for crypsis.

Parasitic cuckoos also showed rates of evolution of egg size that were nine times higher compared with non-parasitic cuckoos. This supports the idea that, in cuckoos, the parasitic breeding strategy probably increased phenotypic diversity between species, at least in traits that have proved to be adaptive in parasites, such as egg size [22]. Nevertheless, we detected no shifts in rates of evolution of egg size for the other lineages. This is unsurprising for Vidua finches, which are less virulent (except for *A. imberbis*) and parasitize very closely related species mostly belonging to the same family and mostly of similar size, so there is little reason to expect diversifying selection. For the honeyguides, we expected higher diversity in egg size, given the evidence showing that they match the egg sizes of several hosts of varying size [18]; however, that study reported major host-specific differences in egg size within the greater honeyguide (*Indicator indicator*), suggesting that high egg size diversity might be occurring at the level of host races rather than at species. Body size did not show increase in rates of evolution associated with the appearance of brood parasitism in any of the three lineages. Interestingly, we also found that the New World cuckoos (genus *Tapera*, *Clamator* and *Dromococcyx*), just like the Old World cuckoos, presented a smaller body size and smaller egg size than non-parasitic cuckoos. However, these three genera did not show changes in rates in any of the traits. This could be owing, however, to the small size of the clades (max three spp.), which makes it harder to detect macroevolutionary patterns.

From the three largest lineages of brood parasites, only the cuckoos (the largest radiation) showed a signal of higher rates of phenotypic evolution. We hypothesized that host breadth could be associated with such differences between lineages. Brood parasites from the three lineages are present in southern Africa, allowing us to compare how wide and diverse the host niche is when the three parasitic lineages are sympatric. Our results partially agree with the expectation that lineages of brood parasites that exploit a higher diversity of hosts (e.g. more distantly related among

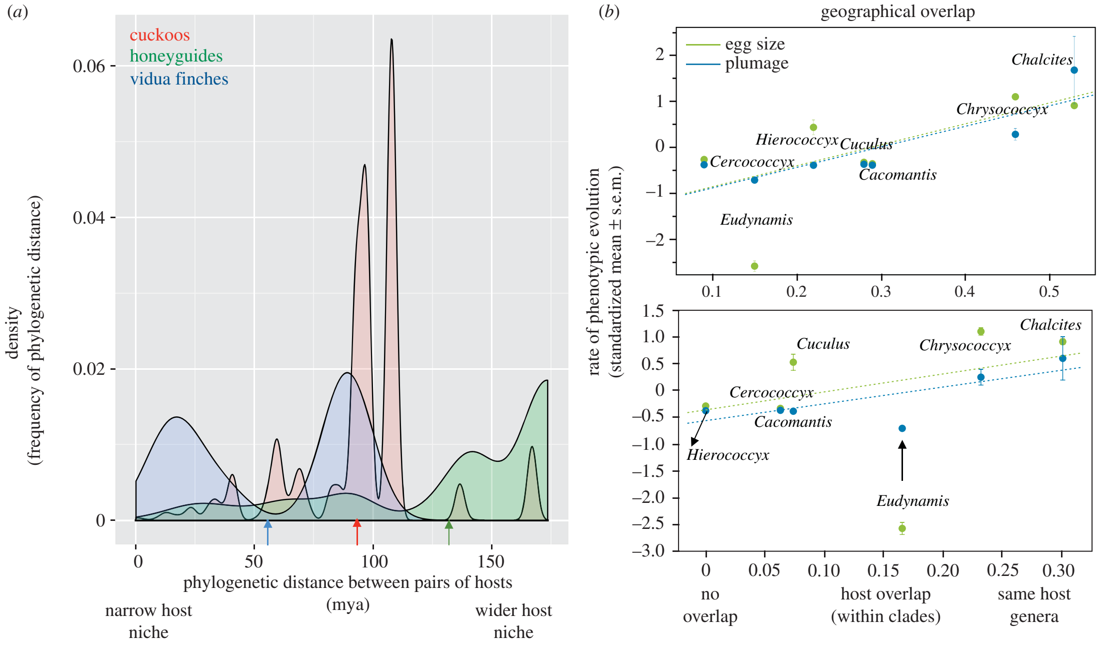


Figure 3. (*a*) Host niche breadth of parasitic cuckoos, honeyguides and Vidua finches. Honeyguides have the highest average breadth, because the lineage parasitizes host species from different taxonomic orders, whereas Vidua finches parasitize species from the same family and thus show a narrow host breadth. (*b*) Relationship between overlap in host use, geographical overlap and rates of evolution of plumage (blue online, dark grey in print) and egg size (green online, light grey in print) within clades of parasitic cuckoos. Parasitic cuckoo clades that overlap more in use of host genera and geographically, show higher rates of phenotypic evolution. (Online version in colour.)

them) experience higher rates of phenotypic evolution. Cuckoos had a wider host breadth and increased evolutionary rates compared with Vidua finches. However, honeyguides do not follow this pattern, and despite having the widest host niche of the three lineages, they show no signs of increased evolutionary rates, and even show a deceleration in rates of plumage evolution.

Within parasitic cuckoos, we explored whether host partitioning (or geographical isolation) could explain differences in rates of phenotypic evolution. We predicted that clades of cuckoos with low overlap in host use would have higher rates of evolution, because selection would favour diversification in traits to parasitize different genera. Our results show the opposite pattern, and cuckoo genera that have species with high geographical overlap and high overlap in host genera presented higher rates of evolution. In fact, it seems that sharing the same host genera with other phylogenetically related parasites is associated with faster phenotypic evolution. It is important to point out that we measured host overlap at the genus level, so a high overlap index does not mean that parasites are sharing the same host species; it rather means that the species they exploit are within the same genus. We suggest that geographical overlap, when combined with overlap in host genera, may lead to competition for hosts between parasitic species. This idea has been specifically suggested for brood parasites in Australia [41]; accordingly, competition may drive the evolution of more finely tuned adaptations than in non-competitive environments, which, in turn, can lead to increased rates of phenotypic evolution [42,43]. Similarly, this could also explain why the genus *Cuculus*, which shows low overlap in host use and, in general, more geographical isolation between species (broad Old World distribution), shows relatively low rates of phenotypic evolution.

In conclusion, we found evidence that the strategy of brood parasitism can affect rates of phenotypic evolution but not necessarily speciation. Our results show that a brood parasitic breeding strategy can lead to divergent evolutionary scenarios, where phenotypic rates may increase, decrease or remain the same, depending on the trait and the ecology of the lineage studied. Brood parasitism seems to have a greater effect on the evolution of egg size and coloration than body size. Moreover, our results support the idea that competition for hosts (or at least evolution in sympatry) may be an important factor leading to increased trait diversity, and we encourage more studies to understand how host use affects phenotypic diversity in host–parasite systems.

Data accessibility. Dataset is available in the Dryad repository http://dx.doi.org/10.5061/dryad.c79vc.

Authors' contributions. I.M. and N.E.L. conceived the study. I.M. collected and analysed data and prepared the figures. I.M and N.E.L wrote the paper.

Competing interests. The authors declare no competing financial interests.

Funding. We received no funding for this study.

Acknowledgements. We thank Marcel Cardillo, David Duchene, Xia Hua, Thomas Wallenius, Matt Symonds and a reviewer for helpful comments and discussions on previous versions of the manuscript and Leo Joseph for access to the ANWC collection. We also thank Daniel Rabosky for guidance with the BAMM analyses during his workshop at the Australian National University.

References

1. Ehrlich PR, Raven PH. 1964 Butterflies and plants: a study in coevolution. *Evolution* **18**, 586–608. (doi:10.2307/2406212)
2. Yoder JB, Nuismer SL. 2010 When does coevolution promote diversification? *Am. Nat.* **176**, 802–817. (doi:10.1086/657048)
3. Rabosky D. 2014 Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* **9**, e89543. (doi:10.1371/journal.pone.0089543)
4. Davies NB. 2000 *Cuckoos, cowbirds and other cheats*. London, UK: T. & A.D. Poyser.
5. Davies NB, Welbergen JA. 2009 Social transmission of a host defense against cuckoo parasitism. *Science* **324**, 1318–1320. (doi:10.1126/science.1172227)
6. Davies NB, de Brooke ML. 1989 An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *J. Anim. Ecol.* **58**, 207–224. (doi:10.2307/4995)
7. Langmore NE, Hunt S, Kilner RM. 2003 Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422**, 157–160. (doi:10.1038/nature01460)
8. Stoddard MC, Stevens M. 2010 Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. B* **277**, 1387–1393. (doi:10.1098/rspb.2009.2018)
9. Langmore NE, Stevens M, Maurer G, Heinsohn R, Hall ML, Peters A, Kilner RM. 2011 Visual mimicry of host nestlings by cuckoos. *Proc. R. Soc. B* **278**, 2455–2463. (doi:10.1098/rspb.2010.2391)
10. Spottiswoode CN, Stevens M. 2012 Host–parasite arms races and rapid changes in bird egg appearance. *Am. Nat.* **179**, 633–648. (doi:10.1086/665031)
11. Payne RB, Payne LL. 1998 *Parasitic birds and their hosts. Studies in coevolution*. Oxford, UK: Oxford University Press.
12. Payne RB. 2005 *The cuckoos*. Oxford, UK: Oxford University Press.
13. Krüger O. 2007 Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Phil. Trans. R. Soc. B* **362**, 1873–1886. (doi:10.1098/rstb.2006.1849)
14. Kilner RM. 2005 The evolution of virulence in brood parasites. *Ornithol. Sci.* **4**, 55–64. (doi:10.2326/osj.4.55)
15. Sorenson MD, Sefc KM, Payne RB. 2003 Speciation by host switch in brood parasitic indigobirds. *Nature* **424**, 928–931. (doi:10.1038/nature01863)
16. Starling M, Heinsohn R, Cockburn A, Langmore NE. 2006 Cryptic gentes revealed in pallid cuckoos *Cuculus pallidus* using reflectance spectrophotometry. *Proc. R. Soc. B* **273**, 1929–1934. (doi:10.1098/rspb.2006.3490)
17. Antonov A, Stokke BG, Vikan JR, Fossøy F, Ranke PS, Røskaft E, Moksnes A, Møller AP, Shykoff JA. 2010 Egg phenotype differentiation in sympatric cuckoo *Cuculus canorus* gentes. *J. Evol. Biol.* **23**, 1170–1182. (doi:10.1111/j.1420-9101.2010.01982.x)
18. Spottiswoode CN, Stryjewski KF, Quader S, Colebrook-Robjent JFR, Sorenson MD. 2011 Ancient host specificity within a single species of brood parasitic bird. *Proc. Natl Acad. Sci. USA* **108**, 17 738–17 742. (doi:10.1073/pnas.1109630108)
19. Krüger O, Sorenson MD, Davies NB. 2009 Does coevolution promote species richness in parasitic cuckoos? *Proc. R. Soc. B* **276**, 3871–3879. (doi:10.1098/rspb.2009.1142)
20. Jetz W, Thomas G, Hartmann A, Mooers O. 2012 The global diversity of birds in space and time. *Nature* **491**, 444–448. (doi:10.1038/nature11631)
21. FitzJohn RG. 2012 Diversitree: comparative phylogenetic analyses of diversification in R. *Methods Ecol. Evol.* **3**, 1084–1092. (doi:10.1111/j.2041-210X.2012.00234.x)
22. Krüger O, Davies NB. 2004 The evolution of egg size in the brood parasitic cuckoos. *Behav. Ecol.* **15**, 210–218. (doi:10.1093/beheco/arg104)
23. Welbergen JA, Davies NB. 2011 A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behav. Ecol.* **22**, 574–579. (doi:10.1093/beheco/arr008)
24. Thorogood R, Davies NB. 2013 Hawk mimicry and the evolution of polymorphic cuckoos. *Chin. Birds* **4**, 39–50. (doi:10.5122/cbirds.2013.0002)
25. Farrell BD. 1998 'Inordinate fondness' explained: why are there so many beetles? *Science* **281**, 555–559. (doi:10.1126/science.281.5376.555)
26. Groot AT, Classen A, Inglis O, Blanco CA, Lopez JJ, Teran-Vargas A, Schal C, Heckel DG, Schofl G. 2011 Genetic differentiation across North America in the generalist moth *Heliothis virescens* and the specialist *H. subflexa*. *Mol. Ecol.* **20**, 2676–2692. (doi:10.1111/j.1365-294X.2011.05129.x)
27. Weber MG, Agrawal AA. 2014 Defense mutualisms enhance plant diversification. *Proc. Natl Acad. Sci. USA* **111**, 16 442–16 447. (doi:10.1073/pnas.1413253111)
28. Krüger O, Davies NB, Sorenson MD. 2007 The evolution of sexual dimorphism in parasitic cuckoos: sexual selection or coevolution? *Proc. R. Soc. B* **274**, 1553–1560. (doi:10.1098/rspb.2007.0281)
29. Krüger O, Davies NB. 2002 The evolution of cuckoo parasitism: a comparative analysis. *Proc. R. Soc. Lond. B* **269**, 375–381. (doi:10.1098/rspb.2001.1887)
30. del Hoyo J, Elliott A, Sargata J, Christie DA, de Juana E. 2014 *Handbook of the birds of the world online*. Barcelona, Spain: Lynx Editions.
31. Schonwetter M. 1967 *Handbuch der oologie*. Berlin, Germany: Akademie.
32. Tarboton W. 2001 *A guide to the nests and eggs of southern African birds*. Cape Town, South Africa: Struik Publishers.
33. Maddison WP, Midford PE, Otto SP. 2007 Estimating a binary character's effect on speciation and extinction. *Syst. Biol.* **56**, 701–710. (doi:10.1080/10635150701607033)
34. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012 Caper: comparative analyses of phylogenetics and evolution in R. R package version 0.5.
35. Rabosky D, Santini F, Eastman JM, Smith SA, Sidlauskas B, Chang J, Alfaro ME. 2013 Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat. Commun.* **4**, 1958. (doi:10.1038/ncomms2958)
36. Hockey PAR, Dean WRJ, Ryan PG. 2005 *Roberts birds of southern Africa*, 7th edn. Cape Town, South Africa: The Trustees of the John Voelcker Bird Book Fund.
37. Kilner RM, Langmore NE. 2011 Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biol. Rev. Cambridge Philos. Soc.* **86**, 836–852. (doi:10.1111/j.1469-185X.2010.00173.x)
38. Fossøy F, Antonov A, Moksnes A, Røskaft E, Vikan JR, Møller AP, Shykoff JA, Stokke BG. 2011 Genetic differentiation among sympatric cuckoo host races: males matter. *Proc. R. Soc. B* **278**, 1639–1645. (doi:10.1098/rspb.2010.2090)
39. Gibbs HL, Sorenson MD, Marchetti K, Brooke MDL, Davies NB, Nakamura H. 2000 Genetic evidence for female host-specific races in the common cuckoo. *Nature* **407**, 183–186. (doi:10.1038/35025058)
40. Trnka A, Grim T. 2013 Color plumage polymorphism and predator mimicry in brood parasites. *Front. Zool.* **10**, 1–10. (doi:10.1186/1742-9994-10-25)
41. Brooker LC, Brooker MG. 1990 Why are cuckoos host specific? *Oikos* **57**, 301–309. (doi:10.2307/3565958)
42. Svanback R, Bolnick DI. 2007 Intraspecific competition drives increased resource use diversity within a natural population. *Proc. R. Soc. B* **274**, 839–844. (doi:10.1098/rspb.2006.0198)
43. Ackerman M, Doebeli M. 2004 Evolution of niche width and adaptive diversification. *Evolution* **58**, 2599–2612. (doi:10.1111/j.0014-3820.2004.tb01614.x)

Synthesis & Conclusions

SYNTHESIS & CONCLUSIONS

Since Darwin (1859), co-evolutionary interactions such as competition, mutualism, parasitism and predation have been suggested to have macro-evolutionary effects and lead to diversification over time. Macro-evolutionary processes are defined as long timescale phenomena that occur at or above the level of species, in contrast to micro-evolutionary processes, which occur within species or populations and over a short timescale (Brooks 1988). Despite the long-standing prediction that coevolution should lead to macro-evolutionary effects (Darwin 1859; Klassen 1992), rigorous exploration of this path has only been possible in recent years, with the development of robust phylogenies and novel phylogenetic methods. Interestingly, there is still limited evidence supporting the role of coevolution in the generation of diversity (Summers et al. 2003; Nunn et al. 2004). In plants, for example, mutualism with pollinators has long been used to explain the huge diversity of angiosperms, but while some studies have supported this claim, such studies involve only 1.4% of all the angiosperm species and there is no consistent pattern (van der Niet and Johnson 2012).

A few years ago, Yoder and Nuismer (2010) used an evolutionary model to propose that, although interactions such as mutualism and predation may lead to arms races, not all types of co-evolutionary interactions necessarily lead to diversification. Specifically, their results show that only in cases of antagonistic interactions, and where there is phenotype matching, will both exploiters and their victims evolve greater phenotypic diversity (Yoder and Nuismer 2010). One, highly tractable example of this type of interaction is brood parasitism. Avian brood parasites lay their eggs in the nest of other species, their hosts, which take care of a foreign chick (Davies 2000). This strategy is very costly to the host, and has led to the evolution of different types of defences, such as rejection of the parasitic egg and mobbing of adult brood parasites (Davies 2000). Egg rejection by hosts has led to the evolution of phenotype matching; rejection of odd-looking eggs by the host selects for parasite eggs that match the size and colouration of their host´s eggs and thereby evade detection (Krüger and Davies

2004; Stoddard and Stevens 2010). These phenotypic changes can occur in as little as 40 years (Spottiswoode and Stevens 2012). Therefore, avian brood parasitism seems to be the ideal system to test whether coevolution can lead to increased diversity in both hosts and parasites. Moreover, brood parasitic interactions may involve few species, contrary to many other host-parasite interactions that involve several parasite species exploiting the same host, making it much easier to isolate the source of selection, identify patterns and test predictions (Rothstein 1990).

In this thesis I investigated the role of brood parasitism in the phenotypic and species diversity of hosts and parasites. Specifically, I explored in different chapters whether brood parasitism was linked with the evolution of variation in egg rejection behaviour, clutch size, cooperative breeding, egg phenotype, breeding phenology and rates of phenotypic evolution. In the next lines I will summarize the results of these studies and discuss these findings collectively.

Coevolution of brood parasites and their hosts

The outcomes of the evolution between two interacting species greatly depend on the costs of the interaction (Yoder and Nuismer 2010). Dawkins and Krebs (1979) illustrate this principle with an example from Aesop's fables:

> *"The rabbit runs faster than the fox, because the rabbit is running for his life while the fox is only running for his dinner." Aesop*

There are strong selective pressures on both hosts and brood parasites. If hosts don't defend they will lose all their progeny and if parasites don't parasitize effectively they won´t reproduce. However, only a few individuals in the host population will be parasitised, but all brood parasites in the population will need to parasitise in order to reproduce. Therefore, selective pressures might be stronger in brood parasites than their hosts. In the next sections I will show evidence of the effects at macro-evolutionary level of brood parasitism in both the exploiters and the victims.

Host evolution

Hosts of avian brood parasites suffer very high costs as a result of parasitism; they invest time and energy feeding a chick that is not their own and in many cases also lose all their progeny ((Davies 2000). More than 500 species of passerines are hosts of brood parasites, in addition to some non-passerines such as woodpeckers and bee-eaters (Friedmann 1955; Friedmann and Kiff 1985; Brooker and Brooker 1989b; Soler 2014).

Hosts have evolved effective defences, such as egg rejection and mobbing, that are specific to the context of brood parasitism, but does brood parasitism also select for the evolution of changes to broader life history traits (or behaviours) besides these, such as changes to mating systems, breeding phenology or reproductive investment? Two new forms of defence were investigated in this thesis.

Firstly, hosts that exhibit cooperative breeding may gain a benefit if larger groups can defend their nests better from brood parasites than a pair. Conversely, brood parasites may also benefit by exploiting cooperatively breeding species, because there are more individuals to provision their young. There is evidence for one species, the superb fairy-wren *Malurus cyaneus*, that helpers at the nest strengthen host defences and lead to lower parasitism rates (Feeney et al. 2013). My comparative analysis (Chapter 4) suggests that this may be a general pattern across cooperative breeders; there was a highly significant correlation between being a cooperative breeder and being a host of a brood parasite, and this pattern is true on the two continents with the largest number of virulent brood parasites: Africa and Australia.

Another potential defence mechanism against parasites is tolerance, where hosts reduce the costs of parasitism by adjusting life-history traits (Svensson and Råberg 2010). Clutch size reduction has been proposed as a tolerance mechanism (Brooker and Brooker 1989a; Hauber 2003). In hosts of parasites like cuckoos (where all the host progeny is lost), species with smaller clutches will lose less progeny when parasitized than hosts with a large clutch (Hauber 2003). In Chapter 2, I explored whether clutch size reduction has evolved as a form of tolerance across host species. By using an analytical model, my collaborators and I found no theoretical support for the evolution of a reduced clutch size in hosts. Moreover, through a comparative analysis we showed that hosts and non-hosts did not have different clutch sizes, after controlling for other variables known to influence clutch size, such as body size, latitude and nest type. This study also shows experimentally that it is unlikely that a small clutch size in hosts evolved as a result of parasites targeting species with smaller clutches. Cuckoo chicks do not benefit from evicting fewer eggs from their host´s nest, and it was equally costly for a cuckoo chick to evict one or five eggs. Despite the importance of brood parasitism in the evolution of other characters, such as cooperative breeding, it does not seem to be linked to the evolution of clutch size.

Brood parasitism is linked to variation in egg phenotype, both within and among host species. Theory suggests that hosts should evolve high variation in egg phenotype between individuals of the same population, since it would be harder for the parasite to mimic different types of eggs (Øien et al. 1995; Soler and Møller 1995). This micro-evolutionary prediction has been supported by many studies (Stokke et al. 2002; Lahti 2005; Landstrom et al. 2010), and we found evidence for the same pattern in Australia (Chapter 7). Moreover, Yoder and Nuismer (2010) specifically suggested that antagonistic interactions with phenotype matching (such as in brood parasites) should lead to phenotypic diversity among host species. For instance, in a microcosm experiment, Buckling & Rainey (2002) showed that the diversity between isolated populations of the bacteria *Pseudomonas fluorescens* was higher in communities with parasites, since *P. fluorescens* populations followed divergent evolutionary trajectories to resist the parasite, resulting in increased diversity. We found a directly analogous result for the hosts of Australian cuckoos (Chapter 7); species that are hosts of brood parasites have diverged more in their egg pattern than those species that are non-hosts, after controlling for time. We propose a similar scenario to that described by Buckling & Rainey (2002), namely, the egg phenotype of each species of host is evolving in response to parasites along a different evolutionary trajectory. Thus, we observe a higher diversity of egg patterns among host species, supporting the predictions of Yoder and Nuismer (2010).

The development of new phylogenetic techniques and robust phylogenies has also allowed us to make use of pre-existing data to explore the evolution of defences from a macro-evolutionary perspective. In Chapter 3, I explored which variables best predict the evolution of egg rejection behaviour in hosts. By collating experiments performed on almost 200 species, this study shows that it is possible to predict which species will evolve egg rejection behaviour, based on whether the parasite is highly virulent (e.g. kills all the progeny of the host) or not, and on the relative size of the host compared to it´s parasite. Hosts that are small relative to the parasite, and are exploited by a highly virulent parasite, are more likely to express egg rejection behaviour.

Although some variables can predict the evolution of egg rejection, it is also an interesting research avenue to understand why some populations lack egg rejection, and how alternative defences evolve. In en empirical study (Chapter 6) I explored whether the evolution of egg rejection is associated with light availability. The yellow-rumped thornbill (*Acanthiza chrysorrhoa*) is the main host of the shining bronze-cuckoo (*Chalcites lucidus*) in Australia, however, egg rejection and chick rejection are very rare in this host. Yellow-rumped thornbills have closed domed nests, which may constrain discrimination of cuckoo eggs and chicks, and therefore the evolution of egg rejection behaviour. We used this system to test experimentally whether increasing the illumination inside the nest affects the likelihood of egg rejection. Contrary to expectations, rejection levels remained very low with increased light levels; moreover, we found very high variation in the natural light levels of the nests, with some nests being as bright as those of other rejecter species with cup nests. These results suggest that other constraints besides light availability may explain the absence of egg rejection in some species.

Given that egg rejection (and chick rejection) rates are very low in yellow-rumped thornbills, in Chapter 5 I explored the evolution of alternative defences. We show that this host has front-line defences, and can recognize and mob cuckoos. Most importantly, we provide evidence that this host breeds earlier than other sympatric and congeneric species, when parasitism rates are lower. We suggest that selection may favour a shift in the breeding phenology of this host in order to avoid the costs of brood parasitism.

Brood parasite evolution

Brood parasites depend exclusively on their hosts to reproduce and this strategy has evolved independently in seven lineages of birds (Payne and Payne 1998). Parasitic cuckoos (Cuculidae, 52 spp.) have a worldwide distribution and constitute the largest radiation, followed by honeyguides (Indicatoridae, 20 spp.) and Vidua finches (Viduidae, 17 spp.), which are exclusively African, cowbirds (*Molothrus spp.*, 6 spp.) and one species of duck (*Heteronetta atricapilla*), which occur in the Americas. These lineages have different strategies; most of the cuckoos and honeyguides kill all the progeny of their host (highly virulent *sensu* Kilner 2005), while chicks of Vidua finches and cowbirds can be raised alongside host progeny (Kilner 2005). Such differences in the cost of the interaction may have macroevolutionary consequences. For instance, in plant-herbivore systems, communities where herbivory is less costly are less diverse than communities where herbivory is very costly (Fornoni 2011). Also, mutualistic interactions, where both participants benefit, are much less likely to lead to diversification (Yoder and Nuismer 2010). Consideration of these differences is important in understanding the role of brood parasitism in diversification processes. For example, an almost mutualistic relationship has evolved between the great spotted cuckoo (*Clamator glandarius*) and it´s host, the carrion crow (*Corvus corone*), where crow chicks benefit from repellent secretions produced by cuckoo chicks, which decreases predation rates (Canestrari et al. 2014). Diversification would not be expected in this type of system.

Although brood parasitism is very costly to hosts, brood parasites are under even stronger selective pressures. In order to reproduce, brood parasites, which are usually much larger than their host, have evolved very small eggs and even body size in order to effectively parasitize their hosts (Krüger and Davies 2004). Moreover, some brood parasites have also evolved plumage that resembles other bird species to avoid detection (Feeney et al. 2015) or to intimidate their hosts (Davies and Welbergen 2009).

In Chapter 8 I explored whether brood parasites are more diverse and have higher rates of phenotypic evolution in traits associated with a parasitic lifestyle. Contrary to expectations (Krüger et al. 2009; Yoder and Nuismer 2010), there is no evidence of increased rates of speciation in avian brood parasites. However, our study shows for the first time that rates of phenotypic evolution are higher in parasitic cuckoos than in non-parasitic cuckoos and other brood parasites. Egg size and a component of plumage associated with brood parasitism evolve faster in parasitic cuckoos, suggesting that brood parasitism can lead to increased phenotypic diversity in the exploiters, as predicted by Yoder and Nuismer (2010). This study also points out that differences in the costs of parasitism and the particular history of the clade make it very hard to generalize predictions. Cuckoos seem to be the only lineage where brood parasitic behaviour is linked to increased diversity, compared to honeyguides and Vidua finches. This is not totally unexpected, given the differences in virulence between the three lineages, the age of the clades, and their contrasting parasitic strategies. Cuckoos parasitise hosts from very distantly related taxa, and constitute the oldest radiation of brood parasites (~65 MYA). Honeyguides also parasitise very distantly related hosts, including passerines and bee-eaters, but they are a much younger lineage (~20 MYA). Finally, Vidua finches are less virulent than both cuckoos and honeyguides, and they are very young clade (~2.5 MYA, Spottiswoode et al. 2011), additionally, this clade parasitises hosts from their sister family, Estrildidae. Therefore, it is not unexpected that Vidua finches did not show any signal of an increase in rates of phenotypic evolution. As suggested by Yoder and Nuismer (2010), not all co-evolutionary interactions are likely to lead to increased diversity, and even within types of interactions, differences in parameters such as the strength of selection determine the likelihood of diversification.

In conclusion, this thesis provides novel evidence that brood parasitism has important macro-evolutionary effects in brood parasites and their hosts, an area that has received relatively little attention. Brood parasitism can be a strong selective pressure, tightly linked to the evolution of important traits such as egg pattern, egg size, plumage, cooperative breeding and breeding phenology, and should be considered an important factor in the evolution of birds. Coevolutionary interactions between brood parasites and their hosts appear to generate phenotypic diversity in both hosts and parasites, mainly in the cuckoo-host system. Hopefully this thesis has paved the way for further research on the macro-evolutionary side of the brood parasitic interaction.

Future directions

1. This thesis has incidentally shown that cuckoos in Australia are a special case. The Australo-papuan genus *Chalcites* has evolved a very small body size compared to other cuckoos, and also shows higher rates of evolution than any other cuckoo genus. Thus the interactions between Australian cuckoos and their hosts are fundamental to understanding rates of speciation in brood parasites and the evolution of tolerance in their hosts.

2. The current availability of robust phylogenies and reliable phylogenetic methods provides the opportunity to test many predictions about brood parasitism, it´s evolution and it´s implications at the macroevolutionary level. For instance, in the last years some parasitism studies in primates (Nunn et al. 2004; Cooper et al. 2012) have incorporated phylogenetic information into analyses of parasite specialization. This avenue that has not been explored in the brood parasite literature (although see Medina & Langmore, in prep), where specialization is often measured simply as the number of host species, without taking into account whether the species belong to the same clade. The evolution of specialization in brood parasites has not been fully explored; it is widely assumed that the evolutionary trajectory is to transition from host generalist to host specialization but there is no rigorous evidence available (Winfree 1999). The main support for this idea is the fact that the oldest lineage of brood parasites (parasitic cuckoos, ~65 MYA), is more specialized than the very young lineage of cowbirds (~3.4 MYA, (Jetz et al. 2012). It would be worth exploring the evolution of specialization in brood parasites from a phylogenetic perspective.

3. Genetic and phenotypic variation among populations have been widely explored in brood parasites (although see Martinez et al. 1999), where several species have evolved host-specific races (Beruldsen 2003; Starling et al. 2006; Spottiswoode et al. 2011). Theory predicts that hosts should also evolve genetic and phenotypic divergence between populations as a consequence of different parasitism regimes (Laine 2009). Recent evidence shows that brood parasitism is linked to host phenotypic diversity at a macro-evolutionary level (chapter 8), and there is ample evidence that parasitism rates (and egg rejection and mobbing) can differ between populations of the same host species (Lindholm and Thomas 2000; Welbergen and Davies 2009). In a review on host-parasite and plant-herbivore systems, Laine (2009) showed that in 100% of the studies, geographic variation in the strength of the interaction has led to the evolution of genetic and phenotypic diversity in hosts. In the case of brood parasites, host populations of the same species that vary in parasitism rates and defences might also be evolving genetic and phenotypic differences, in addition to the observed differences in defences. This possibility has received little attention to date and would be an interesting avenue to explore further.

References

Beruldsen, G. 2003. Australian birds, their nests and eggs, Kenmore hills, Queensland.

Brooker, M. G. and L. C. Brooker. 1989a. The comparative breeding behaviour of two sympatric cuckoos, Horsfield's Bronze-Cuckoo *Chrysococcyx basalis* and the Shining Bronze-Cuckoo *C. lucidus*, in Western Australia: a new model for the evolution of egg morphology and host specificity in avian brood parasites. Ibis 131:528-547.

Brooker, M. G. and L. C. Brooker. 1989b. Cuckoo hosts in Australia. Australian Zoological Reviews 2:1-67.

Brooks, D. R. 1988. Macroevolutionary comparisons of host and parasite phylogenies. Annual Review of Ecology and Systematics 19:235-259.

Buckling, A. and P. B. Rainey. 2002. The role of parasites in sympatric and allopatric host diversification. Nature 420:496-499.

Canestrari, D., D. Bolopo, C. J. Turlings, G. Roder, J. M. Marcos, and V. Baglione. 2014. From parasitism to mutualism: Unexpected interactions between a cuckoo and its host. Science 343:1350-1352.

Cooper, N., R. Griffin, M. Franz, M. Omotayo, and J. Fryxell. 2012. Phylogenetic host specificity and understanding parasite sharing in primates. Ecology Letters 15:1370-1377.

Darwin, C. 1859. On the Origin of Species. Murray, London.

Davies, N. B. 2000. Cuckoos, cowbirds and other cheats. T. & A.D. Poyser, London.

Davies, N. B. and J. A. Welbergen. 2009. Social transmission of a host defense against cuckoo parasitism. Science 324:1318-1320.

Dawkins, R. and J. R. Krebs. 1979. Arms races between and within species. Proceedings of the Royal Society of London, Series B: Biological Sciences 205:489-511.

Feeney, W. E., J. Troscianko, N. E. Langmore, and C. N. Spottiswoode. 2015. Evidence for aggressive mimicry in an adult brood parasitic bird, and generalised defences in its host. . Proceedings of the Royal Society 282:20150795.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Functional Ecology 2011:399-407.

Friedmann, H. 1955. The honeyguides. United States National Museum Bulletin 208:1-292.

Friedmann, H. and L. Kiff. 1985. The parasitic cowbirds and their hosts. Western foundation of vertebrate zoology 2:225-302.

Hauber, M. E. 2003. Interspecific brood parasitism and the evolution of clutch sizes. Evolutionary Ecology Research 5:559-570.

Jetz, W., G. Thomas, A. Hartmann, and O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444-448.

Kilner, R. M. 2005. The evolution of virulence in brood parasites. Ornithological Science 4:55-64.

Klassen, G. J. 1992. Coevolution: A history of the macroevolutionary approach to studying host-parasite associations. The Journal of Parasitology 78:573-587.

Krüger, O. and N. B. Davies. 2004. The evolution of egg size in the brood parasitic cuckoos. Behavioral Ecology 15:210-218.

Krüger, O., M. D. Sorenson, and N. B. Davies. 2009. Does coevolution promote species richness in parasitic cuckoos? Proceedings of the Royal Society B: Biological Sciences 276:3871-3879.

Lahti, D. C. 2005. Evolution of bird eggs in the absence of cuckoo parasitism. Proceedings of the National Academy of Sciences of the United States of America 102:18057-18062.

Laine, A. L. 2009. Role of coevolution in generating biological diversity: spatially divergent selection trajectories. Journal of Experimental Botany 60:2957-2970.

Landstrom, M. T., R. Heinsohn, and N. E. Langmore. 2010. Clutch variation and egg rejection in three hosts of the pallid cuckoo *Cuculus pallidus*. Behaviour 147:19-36.

Lindholm, A. and R. Thomas. 2000. Differences between populations of reed warblers in defences against brood parasitism. Behaviour:25-42.

Medina, I. and N.E. Langmore. In prep. The evolution of specialisation in avian brood parasites.

Nunn, C. L., S. Altizer, W. Sechrest, K. T. Jones, R. A. Barton, and J. L. Gittleman. 2004. Parasites and the evolutionary diversification of primate clades. The American Naturalist 164:S90-S103.

Øien, I. J., A. Moksnes, and E. Roskaft. 1995. Evolution of variation in egg color and marking pattern in European passerines: adaptation in a coevolutionary arms race with the cuckoo, *Cuculus canorus*. Behavioral Ecology 6:166-174.

Payne, R. B. and L. L. Payne. 1998. Parasitic Birds and their Hosts. Studies in Coevolution. Oxford University Press, Oxford.

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

Soler, J. J. and A. Møller. 1995. A comparative analysis of the evolution of variation in appearance of eggs of European passerines in relation to brood parasitism. Behavioural Ecology 7:5.

Soler, M. 2014. Long-term coevolution between avian brood parasites and their hosts. Biological Reviews 89:688-704.

Spottiswoode, C. N. and M. Stevens. 2012. Host-parasite arms races and rapid changes in bird egg appearance. The American Naturalist 179:633-648.

Spottiswoode, C. N., K. F. Stryjewski, S. Quader, J. F. R. Colebrook-Robjent, and M. D. Sorenson. 2011. Ancient host specificity within a single species of brood parasitic bird. Proceedings of the National Academy of Sciences 108:17738-17742.

Starling, M., R. Heinsohn, A. Cockburn, and N. E. Langmore. 2006. Cryptic gentes revealed in pallid cuckoos Cuculus pallidus using reflectance spectrophotometry. Proceedings of the Royal Society B: Biological Sciences 273:1929-1934.

Stoddard, M. C. and M. Stevens. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. Proceedings of the Royal Society B: Biological Sciences 277:1387-1393.

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

Summers, K., S. McKeon, J. Sellars, M. Keusenkothen, J. Morris, D. Gloeckner, C. Pressley, B. Price, and H. Snow. 2003. Parasitic exploitation as an engine of diversity. Biological Reviews 78:639-675.

Svensson, E. I. and L. Råberg. 2010. Resistance and tolerance in animal enemy-victim coevolution. Trends in Ecology & Evolution 25:267-274.

van der Niet, T. and S. D. Johnson. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperns. Trends in Ecology and Evolution 27:353-361.

Welbergen, J. A. and N. B. Davies. 2009. Strategic variation in mobbing as a front line of defense against brood parasitism. Current Biology 19:235-240.

Winfree, R. 1999. Cuckoos, cowbirds and the persistence of brood parasitism. Trends in Ecology and Evolution 14.

Yoder, J. B. and S. L. Nuismer. 2010. When does coevolution promote diversification? The American Naturalist 176:802-817.