Brood parasitism by shiny cowbirds

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Thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

Brood parasitic birds lay eggs amongst the clutches of other species, which then assume all costs of parental care on their behalf. This thesis addresses several puzzles of avian brood parasitism, using field studies and theoretical modelling of the generalist parasite, the shiny cowbird (*Molothrus bonariensis*) and select hosts in Argentina. Key findings and conclusions were:

> High parasitism intensity in a host population can result in a cost to hosts of removing parasite eggs from their clutches, and so help to maintain host's acceptance of parasite eggs in evolutionary equilibrium. The cost is to host egg survival: hosts that remove parasite eggs from the clutch increase the risk that their eggs are destroyed by subsequent parasites that visit the nest.
>
> The principal benefit of mobbing as a front-line defence of hosts may be to reduce egg loss due to parasite attack, rather than prevent parasitism itself.
>
> Differences in the acoustic structure of begging calls between parasites and their host's young can be to the parasite's advantage. Parents provisioned unparasitized broods more during broadcast at the nest of shiny cowbird calls than calls of their own species' chicks, in both a common host and a non-host. The long tremulous quality of a cowbird's call functions analogously to a rapid call rate, thereby exploiting a common provisioning rule of avian parents.
>
> A trade-off for maximum growth in parasite nestlings will variously favour or not favour the evolution of nestmate-killing behaviour, depending on a parasite's abilities, relative to host young, to solicit and attain provisions from host parents. Generalist parasites can encounter both sides of the trade-off in different hosts. Meanwhile, indirect fitness costs are unlikely to constrain the evolution of nestmate-killing in shiny cowbirds, as they rarely, if ever, share the nest with siblings.

ACKNOWLEDGEMENTS

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Beyond this, the many people who have helped to realise this research span three continents.

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My profound thanks go to Juan Carlos Reboreda, who provided generous assistance and cowbird expertise from day one. And equally to all those of Juan's group that welcomed me over the years, tolerated my awkward spanish, shared their friendship, their *mate*, and the joys of life in *las pampas*: Cecilia de Mársico, Vanina Fiorini, Diego Tuero, Romina Scardamaglia, Cynthia Ursino, Mariella Gantchoff, Bettina Mahler and Manuela Chomnalez. Particular thanks must go to Dieguito Masson, who took me under his wing in that first year when things were hardest, and Alicia de la Colina and Fer Furiolo who, on top of everything else, provided language lessons, Christmas dinners and a second family. *Extraño mucho a todos*. Here's hoping I'll be back soon.

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Finally, while it hardly seems an adequate means of acknowledgement, I dedicate this thesis:

To my parents, for a lifetime of love and support,

And to Evan, for sharing in the adventure.

Let us now consider the remarkable habits of certain unnatural parents,
commonly and rightly described as parasitic birds.
- WP Pycraft, 1900

CONTENTS

AUTHOR CONTRIBUTIONS

All work in this thesis is primarily my own.

Four people co-author one or more data chapters, and their contributions are as follows:

Alex Kacelnik contributed his ideas and feedback for all data chapters throughout planning phases, data analysis and manuscript preparation.

In all but one data chapter, Juan Carlos Reboreda contributed his ideas during planning phases, and also his feedback during manuscript preparation of Chapters 2 and 5.

In all but one data chapter, Vanina D. Fiorini participated in data collection in the field and offered advice during this time. The same applies for Diego T. Tuero in Chapter 5.

Chapter 1

General Introduction

General Introduction

The following thesis contributes to our understanding of obligate brood parasitism in birds, principally via field studies with the shiny cowbird (*Molothrus bonariensis*), and two of its common hosts, the chalk-browed mockingbird (*Mimus saturninus*) and the house wren (*Troglodytes aedon*).

In this opening chapter, I first introduce brood parasitism as a subject of study; secondly, I propose some novel research opportunities presented by the shiny cowbird system; and thirdly, I outline the specific aims and structure of the chapters to follow.

1. Why study brood parasitism?

We marvel at the many exquisite behavioural adaptations of animals to their worlds, allowing them to forage and fight, hunt and hide, win mates, build nests, migrate, hibernate and more, in ways that better their chance to survive and reproduce. Occasionally, however, we also observe animals acting in ways that do not seem at all to their benefit. Such behaviours pique our interest: If natural selection favours those traits that increase the fitness of their bearers, how is it that we find, for example, wasps that direct their courtship behaviour at flowers (Schiestl 2005), fish that eat their own offspring (Payne et al. 2002), seabirds that kill their own siblings (Anderson 1990), and some animals that engage in apparently spiteful or altruistic exchanges with non-relatives (West and Gardner 2010)? For the most part, closer inspection reveals such cases not to be true challenges to theory, but nature's subtle ways that we failed at first to grasp. In the asking however, we are often able to shed light both on the evolutionary processes that shape animal's behaviours, and the mechanisms that underlie the behavioural repertoires in question.

It is, at least in part, this research principle that has sustained a long-standing scientific curiosity with obligate brood parasitism. An uncommon reproductive strategy, brood parasitism is known from fish, insects and birds, and has been particularly well-studied in the latter, where it characterizes around 100 species worldwide, or 1% of all birds (Davies 2000). Brood parasitic birds do not build their own nests and care for their own young. Instead, they visit the nests of other species, where they first destroy one or more existing eggs and then lay their own. The owners of these nests (the hosts) subsequently provide all necessary care − incubating, protecting and provisioning the young parasite, even if their own offspring perish in the process. Parasites we imagine profit from delegating to others the costs of parenting. The question here then is why do some birds persist in rearing foreign offspring at their own expense?

As a cursory explanation, we can accept that host parental behaviour simply 'misfires' in an unexpected context where it would be otherwise adaptive. Parasites, after all, are aptly named, and their relationship with hosts is clearly an exploitative one. Recognising host's behaviour as erroneous, however, only highlights the many puzzling aspects of their interactions with parasites. Hosts ought to defend themselves against parasites and many do, but the presence and sophistication of such defences vary greatly both between hosts, and between the various stages in the nesting cycle of any given host. Thus, for example, some reed warblers (*Acrocephalus scirpaceus*) can detect and reject the egg of a common cuckoo (*Cuculus canorus*) from their clutch, but are apparently not equipped to do likewise with a cuckoo chick, despite (at least to human eyes) its monstrously foreign appearance (Davies 2011). Meanwhile, large-billed gerygones (*Gerygone magnirostris*) will reject chicks of their parasite, the little bronze cuckoo (*Chrysococcyx minutillus*), shortly after they hatch, but fail to remove the cuckoo's olive-green egg from amongst their own spotted-white clutch (Sato et al. 2010a). More oddly still, some hosts show no defences whatsoever against either parasite eggs or chicks, even when suffering very high parasitism intensities and incidences.

Further questions arise when we consider that successful parasitism may not rely solely on the evasion of host defences. Many hosts do not strictly attend to parasites as they would their own young, but in fact invest in them relatively *more* care, indicating that young parasites may actively manipulate the behaviour of their host or hosts (Davies 2000, Langmore and Spottiswoode 2012). Like the defences of hosts however, the means by which parasites exploit their hosts are variable between species for reasons that are not always transparent. While some species, for example, remove all competition for resources by killing their host's young shortly after hatching, others tolerate their nestmates and will be typically reared in a mixed-species brood (Kilner 2005).

Host and parasite interactions then are complex, and much remains to be investigated if we are to make sense of the evolutionary scenarios that lead one species to rear the young of another. Along the way, we may hope to shed light on topics as diverse as coevolution (e.g. Dawkins and Krebs 1977, Davies and Brooke 1988, Rothstein 1990, Soler and Soler 2000), speciation (e.g. Sorenson et al. 2003, Spottisewoode et al. 2011), social information transfer (e.g. Davies and Welbergen 2009), vocal ontogeny (e.g. West et al. 1981, West and King 1987), sensory ecology (e.g. Stoddard and Stevens 2011), and parent-offspring communication and conflict (e.g. Kilner et al. 1999, Lichtenstein and Dearborn 2004, Holen and Johnstone 2007), amongst others.

2. Shiny cowbirds and their hosts: novel research opportunities

Brood parasitism in birds has evolved independently in six avian clades: Old World cuckoos (Cuculinae, 54 species), New World cuckoos (Neomorphinae, 3 species), honeyguides (Indicatoridae, 17 species), indigobirds and their allies (Ploceidae, 20 species), cowbirds (Icteridae, 5 species) and the Black-headed duck (*Heteronetta atricapilla,* Anatidae). Those species that breed in the Northern Hemisphere have historically attracted the most research attention, particularly the common cuckoo in its European range (e.g. Chance 1940, Davies and Brooke 1988, Lotem 1993, Marchetti et al. 1998, Kilner et al. 1999, Grim 2007).

Shiny cowbirds occur throughout all of South and Central America and parts of the southern United States, except for regions of dense jungle (Lowther and Post 1999). My opportunity to work with these birds came via a collaboration between Alex Kacelnik, my supervisor here at Oxford's Behavioural Ecology Research Group, and Juan Carlos Reboreda and colleagues at the University of Buenos Aires' Laboratorio de Ecología y Comportamiento Animal. Reboreda's team began work in 1999 at the private reserve El Destino, operated by the Elsa Shaw de Pearson Fundación, and located around 120km south of Buenos Aires on Argentina's east coast. The site is approx. 600ha of well-conserved *pampas* grassland with scattered patches of vegetation and it supports breeding populations of shiny cowbirds and several of their principal hosts. Two of these hosts have so far proven to be particularly amenable to study, on the basis that they have accessible nests and are tolerant of human disturbance - the chalk-browed mockingbird and the house wren. I provide some images and further details of the study site in Figure 1.

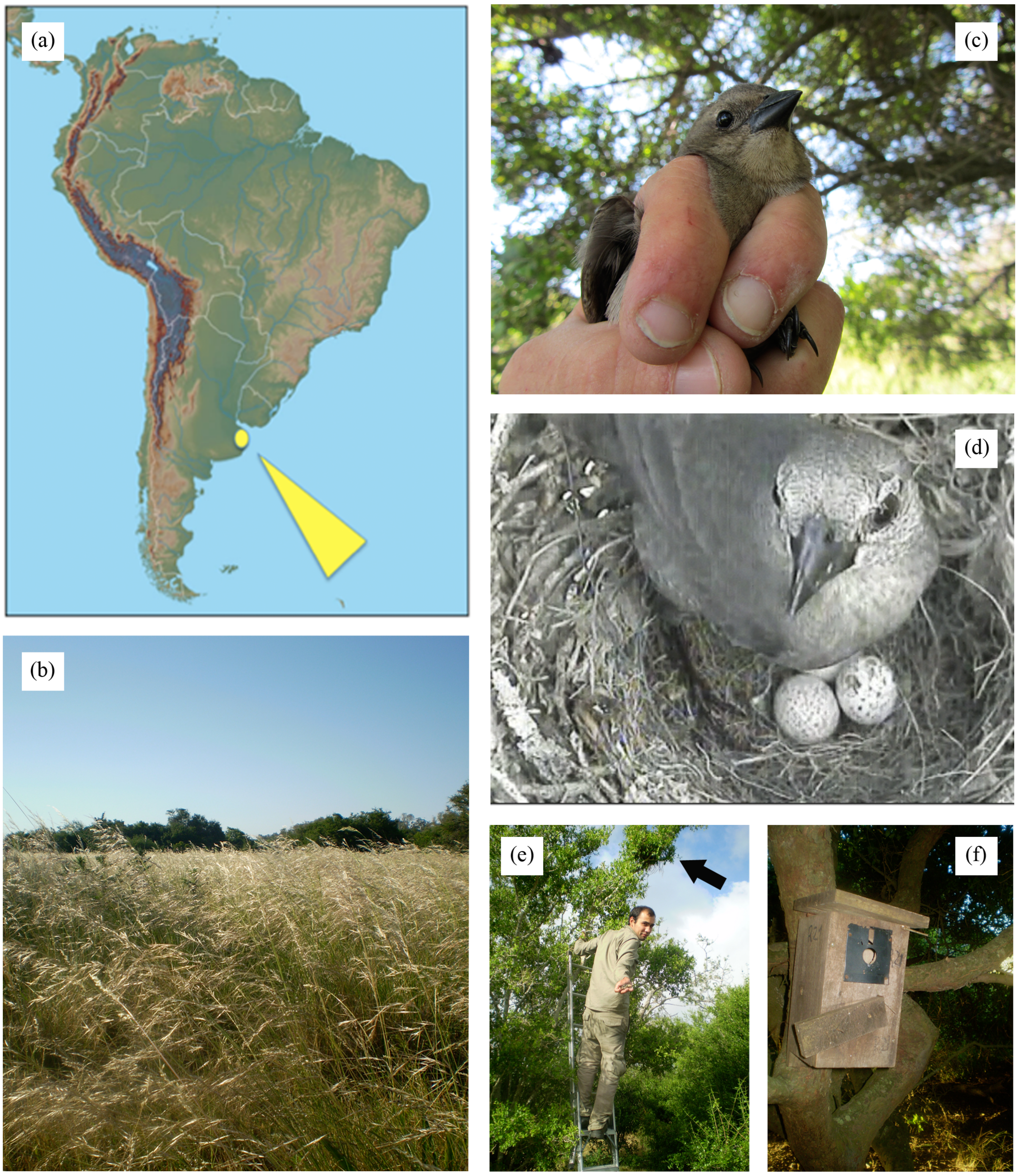

Figure 1. (a) Reserva El Destino is located on the mouth of the Rio de la Plata, Buenos Aires Province, Argentina (35°08′S, 57°23′W); (b) the site is pampas grasslands with patches of trees, predominantly talas (*Celtis tala*), coronillos (*Scutia buxifolia*), and molles (*Schinus longifolius*); here, we have been able to successfully (c) trap and mark female shiny cowbirds, and (d) film them visiting host nests; (e) the open nests of chalk-browed mockingbirds can usually be accessed with a ladder, as my colleague Diego A Masson is shown doing here, an arrow points to the position of the nest; (f) house wrens nest in wooden boxes that we nailed to trees in the interiors of vegetation patches.

Arguably, fieldwork in any under-studied host-brood parasite system has value in that it expands our natural history knowledge. Two prominent aspects of the shiny cowbird system, however, present particular research opportunities beyond that:

(1) *They use many and diverse hosts.*

> Some parasites are specialists, targeting solely or predominantly one host species, and others are generalists (Davies 2000). Shiny cowbirds are at the far end of the generalist spectrum, their eggs or chicks having been observed in upwards of 200 species throughout their range (Lowther and Post 1999). Among the avian parasites, only the brown-headed cowbird (*M. ater*) is known to have a similarly large host catalogue (Lowther 1993). As we might expect from such a long list, shiny cowbird hosts are, furthermore, diverse in their body sizes, life histories and nesting ecologies. Chalk-browed mockingbirds and house wrens exemplify this diversity well. Mockingbirds are open-nesters, nearing twice the size of cowbirds (approx. 75g and 45g respectively), have an incubation period of similar duration (11-12 days vs. 11 days) and their nestlings compete well with cowbirds for food (Fraga 1985). House wrens, in contrast, are cavity-nesters, just one third of the body mass of cowbirds (at just 11-14g), have relatively longer incubation periods (14 days), and their nestlings compete poorly in parasitized broods (Kattan 1996); both hosts are illustrated in Figure 2. It remains unclear whether the shiny cowbird's generalism is manifest only at the population level (i.e. each female targets one host but not all females target the same host; Gibbs et al. 1998), or at the level of individual females (i.e. each female targets multiple hosts, though not necessarily each in equal proportion). The latter may be more likely given that

this is the case in brown-headed cowbirds, their closest relative (Alderson et al. 1999, Woolfenden et al. 2003, Strausberger and Ashley 2005). Either way, the shiny cowbird system presents good opportunities to investigate the implications of generalism for host-parasite evolution.

(2) Hosts are parasitized at high incidences and intensities

Multiple parasitism, in which a nest receives two or more parasite eggs, is commonly observed in the hosts of some parasites, including those of shiny cowbirds (Davies 2000, Ortega 1998). Female cowbirds do not defend territories against other female cowbirds, and nestling cowbirds do not actively attack and kill their nestmates, so multiple cowbird chicks can be reared in any one host nest. The implications of multiple parasitism for hosts and parasites remain largely unexplored, but we might expect it to affect both hosts' defensive strategies and the adaptations of parasites. In a more practical sense, high parasitism incidences and intensities make it possible to study the behaviours of adult parasites in quantitative ways that would otherwise be difficult. At El Destino, chalk-browed mockingbirds endure the highest parasitism intensities, with around 80-90% of all mockingbird nests parasitized, 70% of them multiply. Thus by fixing autonomous microcameras into the vegetation above nests, we can reveal for the first time how female shiny cowbirds behave when they visit nests, and how mockingbirds respond.

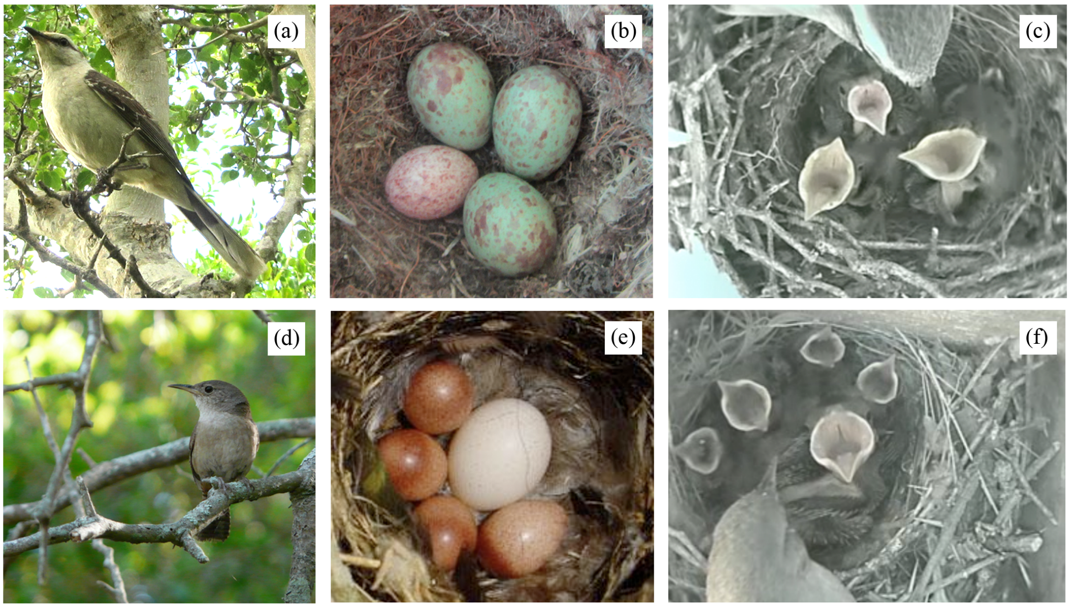

Figure 2. Two common shiny cowbird hosts: top row: (a) a chalk-browed mockingbird; (b) a parasitized mockingbird clutch, in this case containing three mockingbird eggs (larger, green) and one cowbird egg (pinkish), though nests commonly receive multiple cowbird eggs; and (c) a parasitized mockingbird brood, in which the cowbird (top, reddish gape) is smaller than its host nestmates (two larger, yellower gapes). Bottom row: (d) a house wren (14g); (e) a parasitized wren clutch, in this case the wren's eggs are smaller (pink) than the cowbird egg (white); and (f) a parasitized wren brood, in which the cowbird (centre, reddish gape) is larger than its four wren nestmates. Photos (d) and (e) are courtesy of Diego T. Tuero.

3. Aims and structure of this thesis

The broad aims of this thesis then are two-fold: firstly, to address some of the puzzles of avian brood parasitism that can be aptly studied in the shiny cowbird system; and secondly, to advance our basic knowledge of the behaviour and ecology of this particular brood parasite and its hosts.

Through experience, I found that the best returns on time and equipment in the field were gained by pursuing each host nest throughout its development: from eggs (i.e. addressing questions of laying, egg-puncturing and nest defence) to chicks (questions of begging, provisioning and nestling growth). Similarly, work in the field favoured concurrent investigations that took variously the perspective of either the parasite, or one or the other host. This approach produced, inevitably, a less-than-linear set of research questions, but aside from being practical, did have the advantage of helping to sketch a broad picture of host-cowbird interactions.

The following chapters are presented as self-contained manuscripts, and each is intended to be able to be read independently. Four people collaborated in the work of one or more chapters: Alex Kacelnik, Juan Carlos Reboreda, Vanina Fiorini and Diego Tuero (see Author Contributions p.*iv*). The five data chapters fall under four 'umbrella' questions, and are briefly introduced below:

Why don't hosts simply remove parasite eggs from their nests?

Chapter 2 explores how multiple parasitism might influence the evolution of egg rejection as an anti-parasite defence of hosts. The ability to recognize and remove foreign eggs from the nest is a widely documented and effective host defence, but it is absent in some host species (Davies 2000). One explanation is that hosts can incur a cost for removing parasite eggs sufficient to outweigh the benefit (Lotem and Nakamura 1998), and one such cost might be the loss of host eggs. As each female parasite attacks existing eggs in the host's clutch prior to laying her own, any parasite eggs already in the nest could dilute the risk that host eggs are destroyed in subsequent parasite attacks (Sato et al. 2010b). If so, a multiply parasitized host that routinely removes parasite eggs should suffer greater loss of its own eggs than one that keeps the parasite eggs in the clutch. We tested this hypothesis in chalk-browed mockingbirds multiply parasitized by shiny cowbirds.

Is the only goal of front-line anti-parasite defences to prevent parasitism itself?

Chapter 3 considers the benefits to hosts of mobbing female parasites at their nests, a common 'front-line' defence (Sealy et al. 1998, Welbergen and Davies 2009). Mobbing has previously been assumed to benefit hosts by preventing parasites from laying eggs, but it might also function to reduce egg loss during parasite attacks. We determined to what extent mobbing by chalk-browed mockingbirds of shiny cowbirds succeeds in either measure.

Do differences between host and parasite nestling begging signals help or hinder the parasite in securing host care?

Chapter 4 investigates whether a nestling parasite can benefit from a begging call that differs greatly from that of the host's own young. It is already well-established that young parasites benefit from vocalizing more rapidly or more loudly than host chicks, as exaggeration of these call features prompts parents to provision at high rates (Kilner et al. 1999). We focused instead on the acoustic structure of the call (what we might see as a call's 'shape' when it is plotted as frequency vs. time), the effect of which on parental provisioning is largely unknown. While some parasite species mimic their host's begging call, others (including, as we show, shiny cowbirds) produce calls entirely unlike those of host young. We assessed the relative provisioning efforts of house wrens in response to their own species' begging calls, and to cowbird calls. We also assessed whether host responses were consistent with widespread provisioning behaviours in birds by repeating experiments in a ‘parasite-naïve’ non-host, the great tit (*Parus major*). Work with great tits was conducted over two field seasons at Wytham Woods, Oxfordshire, in a nestbox population managed by the Edward Grey Institute for Ornithology, Department of Zoology, University of Oxford.

Why don’t all nestling parasites kill their host’s offspring?

Chapter 5 assesses whether a trade-off for maximum provisions could explain why some nestling parasites kill their host’s offspring shortly after hatching and others do not. Nestmate-killing removes all competition for parental provisioning, and has long been viewed as an adaptive strategy (Davies 2000). Recent studies have proposed however that such ruthless behaviour will not benefit parasites in all cases (Kilner et al. 2004). As host nestmates assist parasites in soliciting parents to bring food to the nest, parasites might in some cases enjoy a higher net food intake when host young are present than when they are absent (Kilner 2005). We formalized this hypothesis theoretically, and tested the outcome of such a trade-off for shiny cowbirds reared in both chalk-browed mockingbirds and house wrens.

Chapter 6 investigates whether indirect fitness costs might prohibit the evolution of nestmate-killing in shiny cowbirds. This would be the case if multiple cowbird eggs in the same nest were laid there by the same female, such that young parasites shared their nests with siblings or half-siblings. We use video recordings of nest visits and egg morphology to assess whether the same or different cowbird females are responsible for multiple parasitism in chalk-browed mockingbirds.

A General Discussion (**Chapter 7**) summarizes the key findings of this thesis and assesses the extent to which its aims were achieved.

References to Electronic Supplementary Materials (ESMs) are made in each chapter, and these files can be found on the accompanying disc.

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Chapter 2

Brood parasite eggs enhance egg survivorship in a multiply parasitized host

Gloag, R., Fiorini, V.A., Reboreda, J. C. & Kacelnik, A.
Proceedings of the Royal Society B 279: 1831-1839

ABSTRACT

Despite the costs to avian parents of rearing brood parasitic offspring, many species do not reject foreign eggs from their nests. We show that where multiple parasitism occurs, rejection itself can be costly, by increasing the risk of host egg loss during subsequent parasite attacks. Chalk-browed mockingbirds (*Mimus saturninus*) are heavily parasitized by shiny cowbirds (*Molothrus bonariensis*), which also puncture eggs in host nests. Mockingbirds struggle to prevent cowbirds puncturing and laying, but seldom remove cowbird eggs once laid. We filmed cowbird visits to nests with manipulated clutch compositions and found that mockingbird eggs were more likely to escape puncture the more cowbird eggs accompanied them in the clutch. A Monte Carlo simulation of this 'dilution effect', comparing virtual hosts that systematically either reject or accept parasite eggs, shows that acceptors enjoy higher egg survivorship than rejecters in host populations where multiple parasitism occurs. For mockingbirds or other hosts in which host nestlings fare well in parasitized broods, this benefit might be sufficient to offset the fitness cost of rearing parasite chicks, making egg acceptance evolutionarily stable. Thus, counter-intuitively, high intensities of parasitism might decrease or even reverse selection pressure for host defence via egg rejection.

1. INTRODUCTION

Some hosts of brood parasitic birds recognise and remove alien eggs from their nests (Rothstein 1990, Winfree 1999). Those that do not might later reject the parasite nestlings directly or abandon the breeding attempt (Langmore et al. 2003, Sato et al. 2010a). In the majority of cases however, ‘acceptor’ hosts will bear the costs of incubating and rearing the young intruders to independence, either in place of their own offspring or in competition with them.

Why egg acceptance prevails in many host species, despite its costs, is a puzzle that has generated several (non-exclusive) hypotheses (Winfree 1999). One line of argument invokes a stepwise co-evolutionary arms race between brood parasites and their hosts in which host defences periodically ‘lag’ behind the adaptations of the parasite (Dawkins and Krebs 1979, Davies 1999, Langmore 2009), evidenced for example in the remarkable egg mimicry evolved by some cuckoos to impede their host’s recognition of foreign eggs (Spottiswoode and Stevens 2011, Stoddard and Stevens 2011). A second general argument is that egg acceptance might be maintained in evolutionary equilibrium if rejection behaviour had fitness costs sufficiently high as to exceed its benefit (Lotem and Nakamura 1998, Takasu 1998, Kruger 2007). A few such costs have been identified; hosts might break their own eggs when attempting to reject a foreign egg (Rohwer et al. 1989, Roskaft et al. 1993), erroneously reject their own eggs (Lotem et al. 1995, Davies et al. 1996), encounter physical constraints (coupled with low renesting success, Kruger 2011), or endure retaliatory nest predation by ‘mafia’ parasites (Soler et al. 1995, Hoover and Robinson 2007).

Figure 1. (a) A chalk-browed mockingbird nest multiply parasitized by shiny cowbirds (smaller, pale-background eggs), (b) a shiny cowbird puncturing eggs in the nest of a mockingbird that already contains cowbird eggs, and (c) a mockingbird egg broken during a cowbird attack, with arrows to mark where the cowbird's beak punctured the shell.

In this study, we report a novel cost of egg rejection that arises when hosts are multiply parasitized. Multiple parasitism, where host nests receive two or more parasite eggs, is common to many avian host-parasite systems (Davies 2000). Each parasitic event is typically preceded by the ruin or removal of some pre-existing eggs in the clutch. Assuming therefore that all eggs have some chance of being targeted, any parasite eggs in the nest would dilute the probability of host egg loss in subsequent parasite attacks. Sato et al. (2010a) postulated that this 'dilution effect' might account for why large-billed gerygones (*Gerygone magnirostris*), the host of an ejector-cuckoo, sometimes reject parasite nestlings but not eggs. The principle of clutch dilution however could be at play in any multiply parasitized host, and might be sufficient to favour egg acceptance in any case where parasitism does not preclude host offspring survival. If so, we find the rather counter-intuitive scenario in which high intensities of parasitism select against the evolution of defensive egg rejection, rather than the reverse (Rothstein 1975, Davies and Brooke 1989, Marchetti 1992, Takasu et al. 1993, Davies et al. 1996).

We studied chalk-browed mockingbirds (*Mimus saturninus:* hereafter 'mockingbird') parasitized by the shiny cowbird (*Molothrus bonariensis*) in Argentina. Mockingbirds are common, larger-bodied hosts of shiny cowbirds (adult masses 70-75g and 40-50g respectively), endure high incidences and intensities of parasitism (De Mársico et al. 2010), and accept nearly all cowbird eggs despite these being different in size, shape and colour to their own (Fraga 1985, Mason 1986); Figure 1. Shiny cowbird females meanwhile are proficient assailants of host clutches, using their beak to puncture holes in eggs and striking at any or all eggs present, with hosts later removing those eggs successfully pierced (see example videos, ESMs 1-3). Thus while mockingbird nestlings fare well in parasitized broods (hatching and fledging at rates akin to those from unparasitized broods, Sackmann and Reboreda 2003, Fiorini 2007), egg losses suffered due to parasitism can be severe (Salvador 1984). We first tested the critical assumption of clutch dilution in this system by filming cowbird puncture attacks and comparing egg losses from clutches with manipulated host-to-parasite egg ratios. If retaining parasite eggs in the nest does indeed reduce the risk of host egg loss, we expect more mockingbird eggs to survive cowbird puncture attacks the more cowbird eggs are present in the nest at the time of the attack. We next simulated the consequences of egg rejection on host egg loss for multiply parasitized hosts, using the mockingbird-shiny cowbird system as a model. With this simulation, we illustrate the trade-off faced by multiply parasitized hosts between preserving their own eggs, and those of their parasite.

2. METHODS

(a) *Study site and field methods*

We monitored the incidence and intensity of parasitism and egg punctures in mockingbird nests over three breeding seasons (2008-2011) at our field site, Reserva El Destino (35 08'S, 57 23'W), Buenos Aires Province, Argentina. Mockingbirds build large open nests of sticks and hair that we located by regularly searching trees in breeding territories. To determine whether mockingbird eggs were less likely to be broken during cowbird puncture attack when accompanied by cowbird eggs in the nest, we conducted an experiment during the 2010-11 season. Most cowbird laying is synchronized with the mockingbird's own laying period (one egg per day, clutch size 4-5, Fiorini et al. 2009), thus nests located during or prior to the start of mockingbird laying were selected for filming. Late in the afternoon on each day of the laying period, we manipulated nest contents by adding or removing fresh eggs such that they contained one of three treatment clutches: (i) one mockingbird egg and three cowbird eggs (1:3 dilution), (ii) one mockingbird egg and one cowbird egg (1:1 dilution) or (iii) one mockingbird egg alone (no dilution). Eggs were marked with permanent markers to allow identification. Strict randomization of treatments with respect to host laying day was not possible because cowbirds' arrival was unpredictable, and also because we avoided removing many eggs at once from clutches as this might have triggered nest abandonment; instead we took departures from random allocation into account for the statistical analysis. Following manipulation of nest contents, we suspended microcameras with infrared lights (Handykam High Resolution CCD Colour) in the vegetation above the nest, connected to digital video recorders with timer record at the base of the tree (Cambox mini-DVR; Lawmate PVR-1000). Nests were filmed continuously from 4.30am-10am the following morning, which spanned the laying period of both cowbird (before sunrise) and mockingbird (post-sunrise, morning). At the end of filming sessions, we visited nests to record any egg breakages. In most cases, any host eggs removed during clutch manipulations were returned to their original nests at the end of the experiment, while some or all cowbird eggs were allocated to other projects. All manipulations were performed under permit according to Argentinian regulations.

(b) *Analysis*

Based on nest checks across all years, we calculated the cumulative frequency distribution (*cdf*) and mean number of parasite eggs received in nests at our field site. To assess whether the probability of a nest receiving a parasite egg was independent of the number of cowbird eggs already present (a property which would affect the pay-offs of clutch dilution), we used a chi-square goodness of fit test of the observed distribution of parasite egg load per nest (1-*cdf*) against the expected values under a geometric probability distribution (i.e. the expected distribution if parasitism events occur independently, Snedecor and Cochran 1989).

Our video recordings captured cowbirds visiting nests and puncturing eggs, both during ‘laying visits’ (defined as pre-sunrise visits, in which puncturing was generally followed by laying) and during later ‘puncturing visits’ (post-sunrise, in which puncturing only occurred). When multiple visits occurred in the same morning it was not possible to ascertain beyond doubt which visit resulted in egg breakage so these recordings were excluded from further analysis (see Results for sample sizes). For those visits that were the sole visit made during the recording session we scored whether or not the cowbird attacked and broke the mockingbird egg during its puncture attack, and/or, if present, cowbird eggs. Egg breakage was determined either from observing on the recording that the hosts’ ate and removed the egg following a cowbird puncture attack, or from nest checks made at the end of each morning’s filming. We then employed forward stepwise logistic regressions to relate the incidence of mockingbird egg loss to the number of accompanying cowbird eggs in the nest. In addition to our predictor of interest (number of cowbird eggs), we included in our analysis three further predictor variables, and their interaction terms. Firstly, the duration of the puncture attack (continuous, secs) was included to control for the fact that some cowbird visits were curtailed by the arrival of the host parents which proceeded to mob the intruder. Puncture attack duration, rather than presence/absence of mobbing itself, was selected as a variable because host mobbing did not necessarily impede a cowbird’s puncturing (see ESM 2-3, and Results for further details). Secondly, because it is not known whether shiny cowbirds sometimes return to the same nest on consecutive days, and if so, whether they adjust their puncturing behavior on return visits, we included in our analysis the day of the mockingbird's laying period on which the visit occurred (day 2, 3 or 4), and the natural parasitism status of the nest prior to the manipulation of nest contents (parasitized or not). In all cases, variables were entered into the model if the resulting reduction in deviance was larger than the critical value of chi-square at $\alpha=0.05$ and *d.f.*=1. Model fits were assessed in two ways; the percentage of real breakages that the model would correctly predict and a proportional measure of reduction in deviance R relative to the null model with intercept only, as used by Peeters & Gardeniers (1998). As a further assessment, we also performed post-hoc univariate analyses for each predictor variable.

Finally, we used Fisher's Exact Tests to compare the proportions of host egg (and when present, parasite egg) loss in each treatment to that expected by chance if the probability of breakage decreases according to the number of other eggs in the clutch (i.e. expected breakage success: 100% for lone eggs, 50% if accompanied by one egg and 25% if accompanied by three eggs). Significant deviations from these values would arise if puncture attacks were biased toward one or the other egg type (Llambías et al. 2006), and/or if eggs frequently resisted puncture when attacked. In the case of parasite eggs, the latter at least is expected to be at play, given that cowbird eggs are structurally adapted to reduce their vulnerability to breakage, being rounder and thicker-shelled than those of their hosts and non-parasitic relatives (Spaw and Rohwer 1987, Rahn et al. 1988, Picman 1989, Brooker and Brooker 1991, Mermoz and Ornelas 2004, Igic et al. 2011). Confidence intervals (95%) of proportions were calculated by the exact method (Zar 1999). All statistical tests were performed in SPSS Statistics 17.0 or MATLAB 7.10.0 (R2010a).

3. RESULTS

(a) *Parasitism intensity*

Of all nests (n=347), 89% were parasitized with one or more cowbird eggs and a further 5% were not parasitized but suffered at least one egg loss from punctures; Figure 2a. Over two thirds of all nests received more than one cowbird egg (69%), one third received more than three cowbird eggs (35%), and around half of those more than five cowbird eggs (16%); Figure 2b. Thus multiple parasitism was the typical condition, with a mean (± s.e.) number of cowbird eggs per nest of 3 ± 0.13. Furthermore, egg counts did not differ significantly from expectation if parasitic events occurred independently of the parasitism status of the clutch (Chi-square goodness of fit test for a geometric distribution, χ^2=12.2, $d.f.$=8 p=0.14), i.e. if already parasitized nests were as likely to receive a cowbird egg as those as-yet unparasitized.

(b) *Clutch dilution and egg loss*

In 172 recordings our cameras captured 130 cowbird visits. On arrival in the nest, cowbirds immediately engaged in egg puncturing in almost every case (121 of 130 visits, 93%), even, in one instance, displacing a mockingbird from atop the nest to do so (ESM 3). During laying visits (pre-sunrise), cowbirds were often mobbed at the nest by mockingbirds, which struck at their heads and eyes and pulled out feathers (on 90 of 109 laying visits; 83%, excluding nests which were abandoned the day of the recording); e.g. ESMs 2-3. While the mean duration of puncture attacks was lower for mobbed visits (mean±s.e., not mobbed: 6.7±1s, mobbed: 3±0.2s, t-test: t_{94}=5.3, p<0.01), mobbing rarely prevented the cowbird laying (84 of 90 mobbed laying visits featured successful lays; 93%) nor did it significantly reduce the chance of a puncture attack occurring (relative to undisturbed laying visits, Chi-square test of independence: χ^2=0.3, *d.f.*=1, *p*=0.6). Furthermore, just 5% of puncturing (post-sunrise) visits were mobbed (1 of 21 to active nests), indicating cowbirds can bypass mockingbird nest defense entirely for such visits (e.g. ESM 1).

During puncture attacks, cowbirds struck at both mockingbird and cowbird eggs; e.g. ESMs 1-3. Of all recorded visits, 57 were to nests containing one of our three treatment clutch compositions and were the sole visit made during the recording. Figure 3 shows the proportion of visits in which a mockingbird egg was broken when accompanied by three, one or no cowbird eggs. A logistic regression model indicated that the odds of a mockingbird egg being broken during a puncture attack were significantly associated to the number of cowbird eggs accompanying it in the nest, and the duration of puncture attacks (*n*=57, *R*=24.3, *d.f.*=2, *p*<0.001) with 73.7% of cases correctly predicted. Host laying day, nest parasitism status and their interactions did not significantly reduce the model's deviance and were not entered in the final model. Likewise, the interaction term for the final predictors (number of cowbird eggs*duration of puncture attack) did not significantly improve the fit of the model, indicating that the time cowbirds had available for puncturing did not significantly alter the effect of clutch dilution. Indeed, a model based solely on number of cowbird eggs was able to predict the fate of mockingbird eggs almost as well as the full model (*n*=57, *R*=14.9, *d.f.*=1, *p*=0.001) with 71.9% of cases correctly predicted.

Univariate analyses were consistent with the regression analysis. By these analyses, the proportion of visits in which mockingbird eggs were broken differed significantly between treatments with different cowbird egg number (Chi-Square Test: χ^2=11.5, *d.f.*=2, *p*=0.003; decreasing with increasing cowbird egg number, Figure 3), and was positively associated to time spent puncturing (*n*=57, *R*=5.1, *d.f.*=1, *p*=0.046, correctly predicted 57.9%), while no between group differences were detected for host laying day (Chi-Square Test: χ^2=0.7, *d.f.*=2, *p*=0.96) or parasitism status at the time of the visit (Chi-Square Test: χ^2=0.1, *d.f.*=1, *p*=0.75).

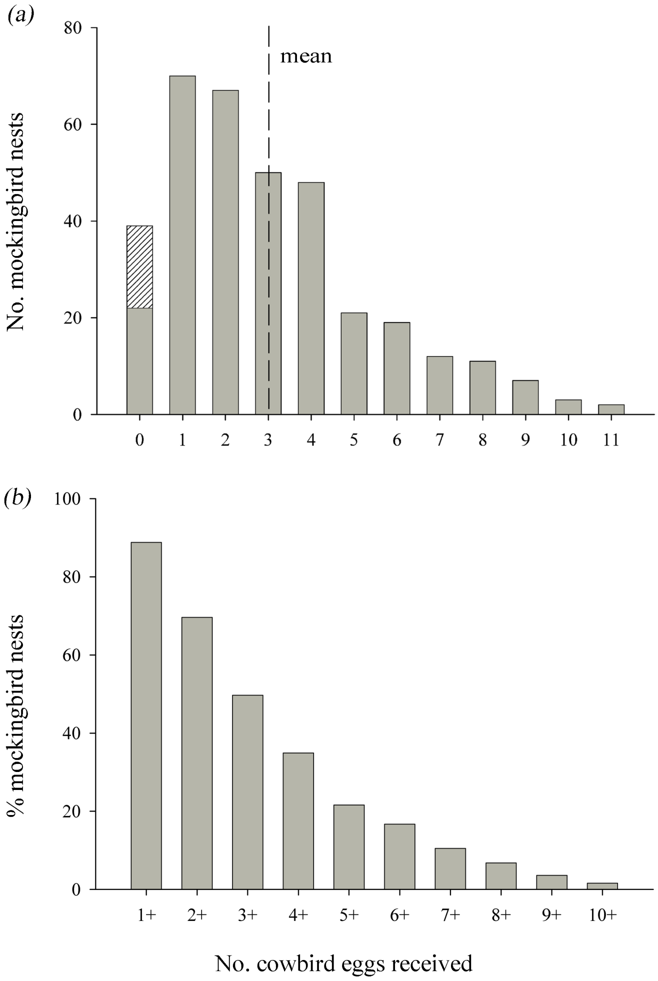


Figure 2. (a) The number of cowbird eggs found in mockingbird nests at our field site during 2008-11 (*n*=347 nests); some nests which were not parasitized nevertheless lost eggs to cowbird puncturing (striped bar). The dotted line indicates the mean. And (b) for the same data, the distribution of nests receiving more than a given number of cowbird eggs (1-*cdf*), which closely approximates a geometric probability distribution, consistent with parasitic events being independent of the parasitism status of the nest at the time they occur.

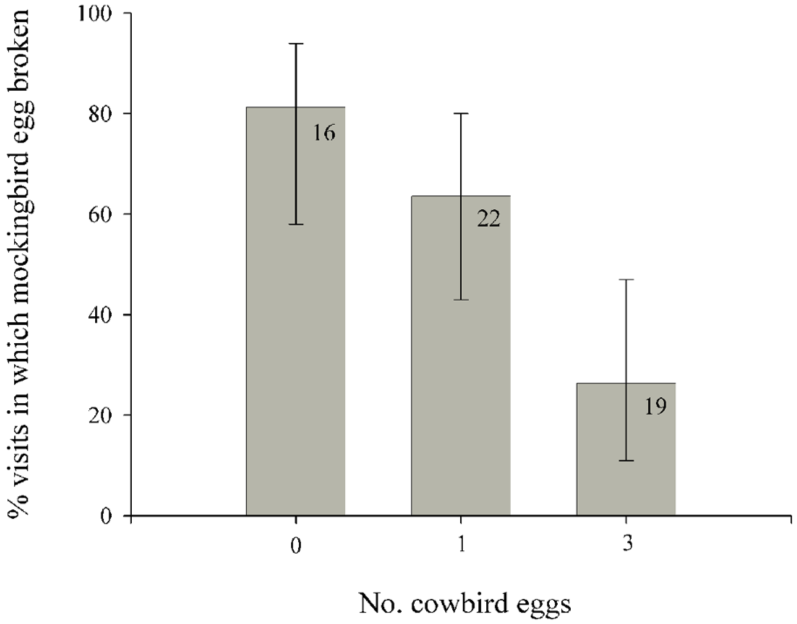


Figure 3. Proportion of cowbird visits (with 95% C.I.) which resulted in mockingbird eggs being broken when the mockingbird egg was the only egg in the nest (number of cowbird eggs = 0) or when accompanied by one or three cowbird eggs at the time of puncture attack. Sample sizes for each treatment group are given inside the bars.

Finally, the numbers of mockingbird eggs lost per treatment did not differ significantly from those expected by chance if the risk of host egg loss decreased according to the strength of clutch dilution (Fisher's Exact Tests: a mockingbird egg alone: p=0.22, a mockingbird egg with one cowbird egg: p=0.54, a mockingbird egg with three cowbird eggs: p=1); thus, if present, any attack bias between egg types did not result in detectable deviations in puncture risk for host eggs. In contrast, cowbird egg loss was less than expected by chance according to clutch composition (one egg broken in 4 of 22 visits when one cowbird egg was present, and one or more egg broken in 6 of 19 visits when three cowbird eggs were present; Fisher's Exact Tests: p=0.03 and p=0.01 respectively).

4. SIMULATION

If the presence of parasite eggs in the nest reduces the risk of host egg damage during parasite visits, then a host that rejects parasite eggs should suffer a decrease in egg survivorship for doing so, provided of course that the rejection occurs prior to the next parasite attack. In rejecter hosts, the majority of egg removal (where eggs are non-mimetic), does occur within a day of parasitism (Sealy 1996, Peer and Sealy 2000, Aviles et al. 2004, Antonov et al. 2007). To assess the consequences of egg rejection under a typical day-by-day regime of parasitism risk, in which host and parasite laying are serially interspersed, we employ a set of Monte Carlo simulations of egg losses and gains from a host clutch. The rules of the simulation can apply to many host-parasite systems, but here we run the models with values appropriate to our mockingbird-shiny cowbird study system.

(a) *Description of the simulation*

We define two categories of host: acceptors and rejecters. The former accept all parasite eggs in the nest. The latter remove any parasite eggs in the nest each day. For the purpose of the simulation, which seeks to isolate the effect of clutch dilution from other costs or constraints of egg rejection, rejecters are assumed to be capable of error-free recognition and rejection. For both acceptor and rejecter, the simulation was run 10000 times for each set of parameters values using MATLAB (Mathworks 2010a). We then calculated the mean number of host eggs surviving in the clutch, H_{ACC} and H_{REJ} for acceptors and rejecters respectively, the difference between these means, H_{DIF} ($=H_{ACC}-H_{REJ}$), and the mean number of parasite eggs surviving in the clutch for acceptors, P_{ACC} ($=P_{DIF}$). H_{DIF} is an index of the cost of rejection i.e. the fitness loss from retaining fewer of one's own eggs, while P_{DIF} is an index of the cost of acceptance i.e. the fitness loss from retaining parasite eggs. In all cases, standard errors of the means were <0.01. The MATLAB script and a glossary of terms are provided in Suppl. Material.

The simulation makes the following assumptions and simplifications:

(i) Hosts lay a clutch of n eggs, one per morning, for n days. The nest is vulnerable to parasite visits for T days where $T=n+1$, that is, for each day during the host's laying cycle plus the first day after host-laying is completed. This captures a scenario by which the majority of parasite laying is synchronized with host laying, while some fraction occurs with a lower likelihood per day during incubation, as is the case for shiny cowbirds parasitizing mockingbirds (Fiorini et al. 2009). Thus, days are 'periods of risk', whereby the final day/period represents the interval between the end of host laying and chicks hatching. For our simulations we fix $T=5$, representing the typical 4-day laying period of mockingbirds.

(ii) On any one day, the probability that at least one cowbird detects the nest is p. Up to two parasites can detect the nest on any one day, where the probability of a second parasite detecting the nest on the same day is p/a and a is a constant ($a \geq 1$). For our simulations, we fix $a=5$ which approximates the ratio between double and single parasitism per day we observed in mockingbird nests from our field study (18 of 87 recordings with at least one laying visit featured a second laying visit).

(iii) Each cowbird that detects a nest makes two visits. On the day of detection, she makes a puncturing visit in which she attacks any eggs present. On the subsequent day the same cowbird returns to the nest for a laying visit, during which she attacks eggs and then lays her own. Figure 4 illustrates the simulation's schedule of parasite visits and host laying (and egg rejection, in the case of rejecters) across days.

(iv) Whether a particular egg is attacked is determined by the ratio of eggs in the nest at the time of the parasite's visit, where H and P are the number of host and parasite eggs respectively. This implements the dilution effect, whereby retention of parasite eggs reduces the risk of host egg loss. Deviations from random in the risk of attack are expressed by *b*, where $b>1$ indicates an attack bias toward parasite eggs, $0<b<1$ indicates bias towards host eggs, and $b=1$ indicates no bias. Thus the probability that any one host egg is attacked, d_H, is given by:

$$d_H = \frac{1}{H} \cdot \left(\frac{H}{H + P.b} \right) = \frac{1}{H + P.b}$$

And likewise the probability that any one parasite egg is attacked, d_P:

$$d_P = \frac{1}{P} \cdot \left(\frac{P.b}{H + P.b} \right) = \frac{1}{\frac{H}{b} + P}$$

(v) Finally, host and parasite eggs when attacked are broken with probability s_H and s_P respectively. Under the simplest scenario, all eggs are equally vulnerable to breakage and $s_H=s_P$. In practice, parasite eggs are likely less vulnerable to breakage than host eggs ($s_P < s_H$), given their structural adaptations (Spaw and Rohwer 1987, Rahn et al. 1988, Picman 1989, Mermoz and Ornelas 2004). Virtual hosts immediately remove broken eggs from the nest following a successful puncture and never abandon the nest during the laying cycle, even if puncturing reduces the clutch to zero.

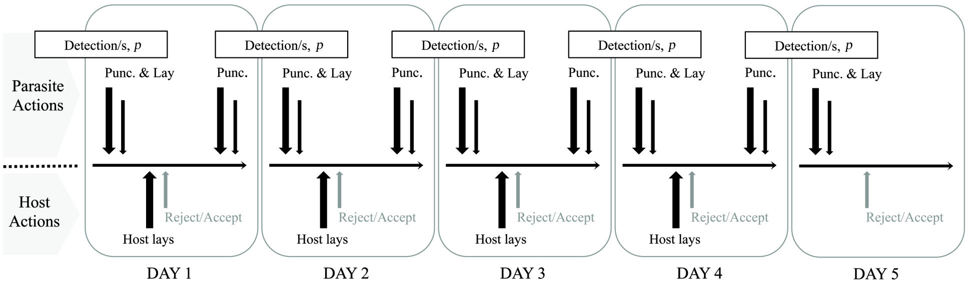
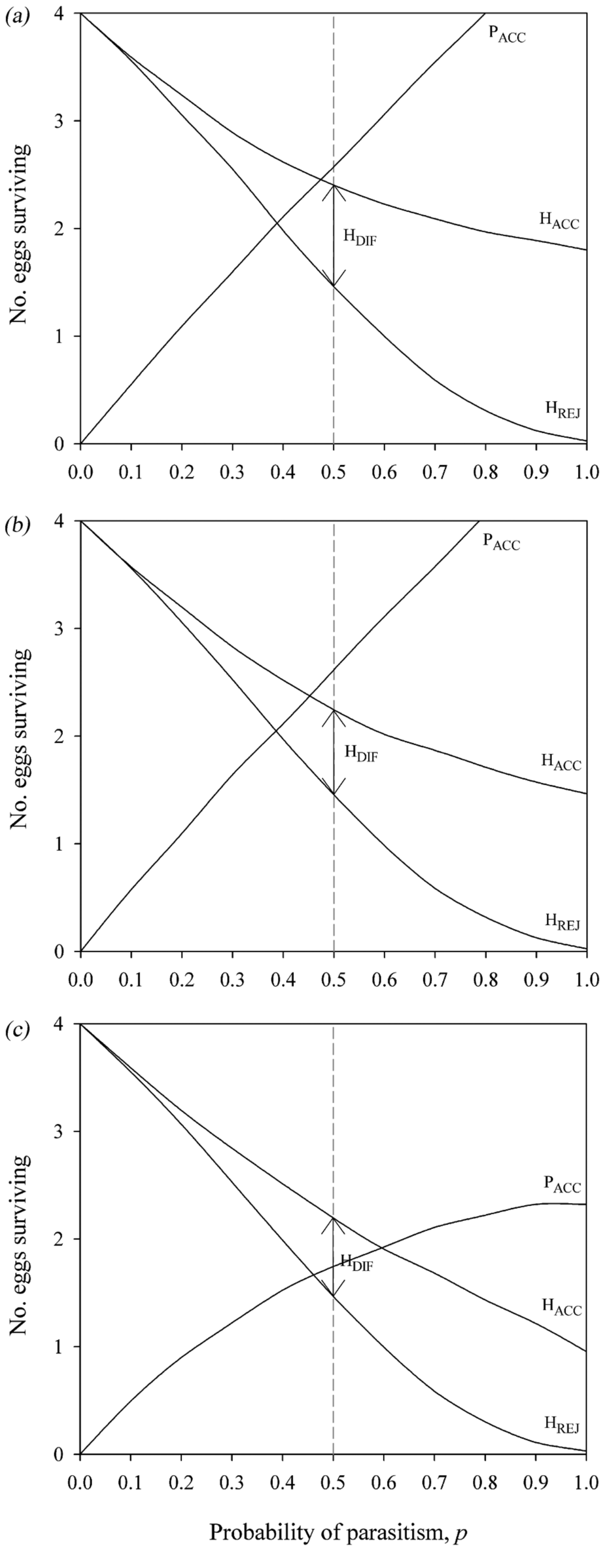


Figure 4. A simulation of multiple parasitism at host nests. Parasite actions are given in the top half of the diagram. Each parasite that detects a nest makes a puncturing visit and a laying visit the following morning during which both puncturing and laying occur. The puncturing visits that precede Day 1 laying visits are not shown as the nest contains no eggs at this time. A maximum of two parasites can detect the nest on any one day (represented by two arrows) and the probability of detection per day is *p*. Host actions are given in the lower half of the diagram. Hosts lay once per day. At the same time each day, they either reject or accept any parasite eggs in the nest, depending on the strategy being simulated. At the end of the final day, we calculate the number of host and parasite eggs in the nest for rejecters and acceptors.

Figure 5. The consequences to egg survivorship of rejection or acceptance of parasite eggs by hosts for increasing probabilities of parasitism, p: the mean number of host eggs surviving for acceptors, H_{ACC}, and rejecters, H_{REJ}, the difference between which is H_{DIF}; and the mean number of parasite eggs surviving for acceptors, P_{ACC}. The intensity of parasitism by shiny cowbirds observed for mockingbirds at our field site is indicated by a dotted line (p=0.5). Simulations were run with three variations: (a) parasite eggs less likely to be broken if attacked than host eggs and risk of attack equal for all eggs, s_H=0.9 and s_P=0.3, b=1 (b) parasite eggs less likely to be broken if attacked and less likely to be attacked than host eggs, s_H=0.9, s_P=0.3, b=0.7, and (c) all eggs equally likely to be attacked and broken if attacked, s_H=0.9, s_P=0.9, b=1.


(a)
(b)
(c)
No. eggs surviving
0
1
2
3
4
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
P_ACC
H_ACC
H_DIF
H_REJ
Probability of parasitism, p

(**b**) ***Egg survival: acceptors vs. rejecters***

Figure 5 shows the results of the simulations (P_{ACC}, H_{ACC} and H_{REJ}) as a function of p, the probability of parasite detection per day, for three illustrative combinations of s and b. In every case, as p increases, and with it the likelihood of multiple parasitism, acceptors enjoy increasingly higher own egg survivorship than rejecters (H_{DIF}); that is, there is a penalty for egg rejection when multiple parasitism occurs.

Figure 5a shows egg survivorships when risk is apportioned equally between all eggs during a cowbird attack (b=1), and parasite eggs better resist puncture than host eggs (here, 90% of host eggs are broken when attacked, s_H=0.9, and 30% of parasite eggs s_P=0.3). A p value of around 0.5 approximates the parasitism intensity endured by mockingbirds at our field site, corresponding to a mean (±SD) number of parasite eggs received per nest in the virtual host population of 3 (±1.4). At this probability of parasitism, from a clutch of four own eggs, an acceptor mockingbird can expect to retain on average one extra egg per clutch than a rejecter mockingbird that consistently removes foreign eggs each day they appear (H_{DIF}=0.95); Figure 5a. Any attack bias towards host eggs, as a result of either their larger size or some discriminatory behaviour by cowbirds would lower H_{DIF}, though acceptors nevertheless retain positive margins of host egg survival over rejecters, as illustrated in Figure 5b (here b=0.7; at p=0.5, H_{DIF}=0.8). Reciprocally, any attack bias towards parasite eggs would shift an acceptor's benefit in egg survivorship even higher (see Suppl. Material for a sensitivity analysis of b).

Low vulnerability of parasite eggs to puncture leads to high survivorship of parasite eggs in acceptor's nests (e.g. at p=0.5, P_{DIF}=2.4; Figure 5a). Figure 5c shows that, in contrast, where host and parasite eggs are equally vulnerable to breakage (90% of all eggs attacked are successfully broken, s_H=s_P=0.9), parasites often break eggs laid by preceding parasites, curbing the rise of parasite egg numbers as parasitism intensity increases (e.g. at p=0.5, P_{DIF}=1.7). Interestingly however, while tough-to-break parasite eggs produce a greater burden of parasite eggs to rear, they also cause a more powerful clutch dilution effect, and thus more pronounced egg survival benefit to acceptors (Figure 5a,c).

In any case, it is the relationship between H_{DIF} and P_{DIF} that will determine the fitness trade-off for hosts between egg acceptance and rejection. The fitness difference, at any value of p, could be represented as H_{DIF} - R(P_{DIF}), where R is a growing (positive first derivative) function describing the loss to a host's reproductive output caused by rearing a given number of parasite nestlings. Simply put, when all else is equal, egg acceptance would be favoured over egg rejection when the discrepancy in host egg survival (H_{DIF}) adds more to a host's fitness than the difference in parasite egg survival (P_{DIF}) detracts from it.

5. DISCUSSION

We show, both in a field experiment and by simulation, that the presence of parasite eggs in a nest dilutes the risk of host egg loss in subsequent parasite attacks. At our field site in Argentina, chalk-browed mockingbirds are multiply parasitized by shiny cowbirds and highly vulnerable to cowbird puncture attacks, despite aggressive nest defence. Video recordings of shiny cowbirds visiting mockingbird nests with experimentally manipulated clutch compositions revealed that mockingbird eggs were more likely to survive a puncture attack the more cowbird eggs were present in the clutch, thereby supporting the critical assumption of clutch dilution in this system. This dilution effect would impose a cost of egg rejection wherever rejection precedes subsequent cowbird visits: as our Monte Carlo simulation shows, when multiple parasitism is common, mockingbirds, or any other hosts, would enjoy a higher mean survival of their own eggs by accepting all parasite eggs than by removing them.

Could this cost of egg rejection be sufficient to make acceptance of parasite eggs evolutionarily stable? We propose at least three conditions would need to be met; i) the host population endures a high frequency and intensity of parasitism (and associated attacks on eggs), (ii) host young can fledge in parasitized broods, and (iii) the residual costs of rearing parasites to a host parent's future reproduction are not higher than the gains from reduced egg mortality. The first condition is made clear from our simulations, with the gap in egg survivorship between acceptors and rejecters widening as the chances of multiple parasite visits increase. Multiple parasitism is commonplace among hosts of Vidua finches (Davies 2000), non-evicting cuckoos (Arias de Reyna 1998) and the South and Central American cowbirds. In the case of cowbirds, Ortega (1998) summarizes reports of parasitism intensities and suggests that multiple parasitism may be the rule for at least some hosts of each of the bronzed cowbird (*M. aeneus*), giant cowbird (*M. oryzivorus*), screaming cowbird (*M. rufoaxillaris*) and shiny cowbird, with the latter appearing to have the highest scores. Parasitism by cowbirds may also be particularly damaging because, unlike some other brood parasites, they commonly make both pre-laying visits and laying visits and may attack eggs on both (Ortega 1998, Astié and Reboreda 2006).

The latter two conditions needed to favour egg acceptance concern the trade-off facing multiply parasitized hosts. Assuming no nestling rejection occurs, the same cowbird eggs that buffer host offspring against destruction at the egg stage will hatch to produce young parasites that compete with host offspring at the nestling stage (a cost to the current brood) and may reduce a host parent's ability to rear subsequent offspring (a cost to future broods, Trivers 1974a). Clearly, where parasite eggs greatly compromise the survival of host young, no amount of clutch dilution will tip the balance in favor of egg acceptance. This includes any negative effects that parasite eggs may have on host egg incubation and hatchability (Hauber 2003, Hoover 2003). However, the mortality of host offspring in parasitized broods varies greatly between cowbird hosts (Ortega 1998, Kilner 2003), and hosts of brood parasites generally (Davies 2000). Costs to the current brood of rearing parasites are likely to be lowest where hosts are cooperative breeders able to enlist helpers in rearing parasitized broods and so reduce the severity of food competition between host and parasite nestmates (Fraga 1998, Canestrari et al. 2009), or where host eggs and/or nestlings are larger than their parasites by virtue of incubation period, chick growth rates or body size. Hosts may even reject parasite nestlings, removing the costs of rearing altogether (Sato et al. 2010a, b). In the case of mockingbirds, host nestlings fare well in parasitized nests, hatching at the same time or one day later than their cowbird nestmates, receiving an equal or greater share of provisioning and growing larger than them within a few days (Gloag et al. 2011), to the extent that their hatching and fledging successes are similar to those of unparasitized broods (Sackmann and Reboreda 2003, Fiorini 2007).

The parental care cost incurred by parents that tend parasitized broods is more difficult to estimate. Any time and energy a host parent invests in caring for parasite chicks should lead to some fractional decline in their ability to invest in other components of their fitness, and thus in future offspring (Trivers 1974a, b). This cost presumably depends on the life history of a host species, including the maximum number of broods in a parent’s lifetime, the risk of parental mortality between one brood and the next, and the rate at which investment increases with increasing brood mass. Again, the magnitude of the cost is likely to be lower when hosts are cooperative breeders, or are relatively large in body size compared to their parasites, because smaller hosts must work harder to rear parasite chicks. Even so, several studies on small-bodied hosts of brown-headed cowbirds (*M. ater*) have failed to detect an effect of parasitism on parents' subsequent survival and nesting success (Smith 1981, Payne and Payne 1998, Sedgwick and Iko 1999, Hoover 2003) illustrating how difficult such effects are to assess. In the case of mockingbirds parasitized by shiny cowbirds, we have no quantitative estimates of the residual rearing cost for parents, nor of the relative weight of this cost in a trade-off between accepting and rejecting cowbird eggs. Fraga (1985) suggested that, like other Mimids, chalk-browed mockingbirds may be brood-reducers, preferentially provisioning the larger chicks so that the smallest one or two may perish before fledging (Fraga 1985). Such a provisioning strategy would mitigate the costs of parental investment for acceptors saddled with larger broods.

Interestingly, mockingbirds are atypical among cowbird hosts in that many individuals do demonstrate some egg rejection, removing pure-white shiny cowbird eggs (Mason 1986), a morph laid infrequently in their nests (<10%; R. Gloag, unpubl. data), while accepting all other eggs. Sackmann & Reboreda (2003) observed that these rejections occurred rapidly, on the first visit host parents made to nests after a white egg was added, and we similarly observed two such rejections in our videos for this study. If clutch dilution procured a net benefit to acceptors, we would expect these morphs to be accepted like all others unless there were special circumstances, such as white eggs attracting a lower rate of attack during puncturing, or being otherwise disproportionately costly to accept. For open-nesting species like mockingbirds, white eggs may, for example, increase nest detectability by predators (Mason and Rothstein 1987). In any case, the full range of costs and benefits of egg rejection for mockingbirds are unknown. Thus while we can confirm a clutch dilution effect is at play in this host, it may be, under the evolutionary equilibrium hypothesis, just one component operating within a larger fitness trade-off that favours egg acceptance.

For hosts of cowbirds and other ‘nestmate-tolerant’ parasites, multiple parasitism increases the load of parasite chicks and thus the costs to hosts of rearing parasitized broods, relative to single parasitism. It would thus be reasonable to expect that an increasing incidence of parasitism would increase selection pressure on hosts to evolve effective anti-parasite defences (Rothstein 1975, Davies and Brooke 1989, Marchetti 1992, Takasu et al. 1993, Davies et al. 1996). Interestingly, the clutch dilution hypothesis contradicts this intuitive expectation with respect to egg rejection. That is, egg rejection can result in greater host egg losses as the probability of parasitism, and so of multiple parasitism, increases. Thus we find that at high intensities of parasitism, the trade-off between egg survival and parasite rearing might not favour defensive egg rejection, but in fact select for the reverse.

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8. SUPPLEMENTARY MATERIAL

(a) *Simulation glossary*

Table S1. Definition of terms used in the simulation

Term	Min	Max	Definition
H_{ACC}	0	T-1	mean no. host eggs surviving for an acceptor after 10000 simulation runs
P_{ACC}	0	T*2	mean no. parasite eggs surviving for an acceptor after 10000 simulation runs
H_{REJ}	0	T-1	mean no. host eggs surviving for a rejecter after 10000 simulation runs
H_{DIF}	0	T-1	difference in mean host egg survivorship for an acceptor and a rejecter; absolute value of H_{ACC}-H_{REJ}
P_{DIF}	0	T*2	difference in mean parasite egg survivorship for an acceptor and a rejecter; =P_{ACC}
n	1	integer	no. eggs in a host clutch
T	0	integer	no. days during which parasites can detect host nest
p	0	1	probability per day of parasite detection
a	1	integer	fraction of parasite detections followed by 2nd parasite
b	0	integer	bias towards striking parasite eggs in the nest
d_H	0	1	probability that a host egg is attacked
d_P	0	1	probability that a parasite egg is attacked
s_H	0	1	probability that a host egg is broken if attacked
s_P	0	1	probability that a parasite egg is broken if attacked

(b) *Simulation script*

A Monte Carlo simulation of host and parasite egg survival for acceptor and rejecter hosts facing increasing probability of parasitism: In the simulation, whether a probabilistic event will occur or not is determined by drawing random samples ($0 \le x \le 1$) from a uniform distribution. For any probability k, if $x \le k$ the event occurs, otherwise it does not. This process applies for each probabilistic variable (p, s and d) on each day of the simulation.

The following script was used to run our simulation in MATLAB (Mathworks 2010a). Annotations for the script are made in green following '%' symbols. The simulation was run once for acceptor and once for rejecter (with 10000 replicates per run) for each probability of parasitism, p (for given values of s_H, s_P and b). The script below is that used for the rejecter, which removes all parasite eggs from the nest. The script for the acceptor is identical except that rejection does not occur.

```
H=0; % number of host eggs to start
P=0; % number of parasite eggs to start
T=[1:1:5]; % number of days of exposure to parasite detection
p=[0:0.1:1]; %probability per day of at least one parasite detection
a=5; %parameter fixing risk of same-day multiple parasitism
sh=0.9; %probability of host egg breaking if struck
sp=0.9; %probability of parasite egg breaking if struck
b=1; %bias towards striking parasite eggs


for j=1:length(p) %start loop for a given probability of parasite detection.

       for k=1:10000 %start replicate run for 'REJECTOR'

       x(i)=random('unif',0,1); %nest detection by one parasite on day 0?
       y(i)=random('unif',0,1); %nest detection by 2nd parasite on day 0?

       for i=1:length(T) %loop for successive days

              if x(i)<p(j) %does one laying visit occur?

                dh=(H/(H+b*P))*1/H;  %probability each host egg attacked
                dp=(b*P/(H+b*P))*1/P;%probability each parasite egg attacked
                BreakH=0; %initialize counter of host eggs broken this visit
                BreakP=0;%initialize counter parasite eggs broken this visit

               %begin loop through host eggs
                if H>0
```

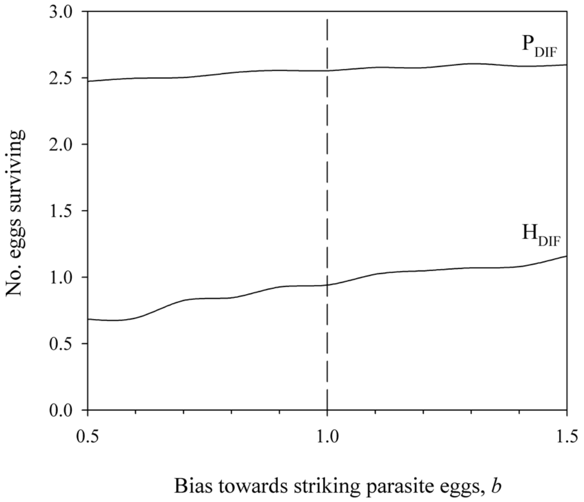
```
    for EggH=1:length(H) %loop through host eggs present
        if random('unif',0,1)<dh(EggH) %is this egg attacked?
            if random('unif',0,1)<sh(EggH) %is this egg broken?
               BreakH=BreakH+1; %update counter
            end
        end
    end
    end
    %end loop through host eggs

   %begin run through parasite eggs
    if P>0
    for EggP=1:length(P) %determine if each parasite egg broken
        if random('unif',0,1)<dp(EggP) %is this egg attacked?
            if random('unif',0,1)<sp(EggP) %is this egg broken?
               BreakP=BreakP+1; %update counter
            end
        end
    end
    end
    %end run through parasite eggs

    H=H-BreakH; %update number of host eggs remaining
    P=P-BreakP; %update number of parasite eggs remaining
    P=P+1; %parasite lays an egg

    if y(i)<p(j)/a %does a second laying visit occur?

    dh=(H/(H+b*P))*1/H;
    dp=(b*P/(H+b*P))*1/P;
    BreakH=0;
    BreakP=0;

    %begin loop through host eggs
    if H>0
    for EggH=1:length(H)
        if random('unif',0,1)<dh(EggH)
            if random('unif',0,1)<sh(EggH)
               BreakH=BreakH+1;
            end
        end
    end
    end
   %end loop through host eggs

   %begin loop through parasite eggs
    if P>0
    for EggP=1:length(P)
        if random('unif',0,1)<dp(EggP)
            if random('unif',0,1)<sp(EggP)
               BreakP=BreakP+1;
            end
        end
    end
    end
   %end loop through parasite eggs

    H=H-BreakH; %update number of host eggs remaining
    P=P-BreakP; %update number of parasite eggs remaining
    P=P+1; %parasite lays an egg
end

P=0; %host rejects all parasite eggs in the nest

if i<=(T-1)

H=H+1; %host lays an egg

x(i)=random('unif',0,1); %nest detection by 1 parasite on day i?
y(i)=random('unif',0,1);%nest detection 2nd parasite on day i?
```

```
if x(i)<p(j) %does one puncturing (non-laying) visit occur?

    dh=(H/(H+b*P))*1/H;
    dp=(b*P/(H+b*P))*1/P;
    BreakH=0;
    BreakP=0;

    %begin loop through host eggs
    if H>0
    for EggH=1:length(H)
        if random('unif',0,1)<dh(EggH)
            if random('unif',0,1)<sh(EggH)
               BreakH=BreakH+1;
            end
        end
    end
    end
    %end loop through host eggs

    %begin loop through parasite eggs
    if P>0
    for EggP=1:length(P)
        if random('unif',0,1)<dp(EggP)
            if random('unif',0,1)<sp(EggP)
               BreakP=BreakP+1;
            end
        end
    end
    end
    %end loop through parasite eggs

    H=H-BreakH; %update number of host eggs remaining
    P=P-BreakP; %update number of parasite eggs remaining

    if y(i)<p(j)/a %does a second puncturing visit occur?

    dh=(H/(H+b*P))*1/H;
    dp=(b*P/(H+b*P))*1/P;
    BreakH=0;
    BreakP=0;

    %begin loop through host eggs
    if H>0
    for EggH=1:length(H)
        if random('unif',0,1)<dh(EggH)
            if random('unif',0,1)<sh(EggH)
               BreakH=BreakH+1;
            end
        end
    end
    end
    %end loop through host eggs

    %begin loop through parasite eggs
    if P>0
    for EggP=1:length(P)
        if random('unif',0,1)<dp(EggP)
            if random('unif',0,1)<sp(EggP)
               BreakP=BreakP+1;
            end
        end
    end
    end
    %end loop through parasite eggs

    H=H-BreakH; %update number of host eggs remaining
    P=P-BreakP; %update number of parasite eggs remaining

    end
```

```
            end
           end
      end %end loop through days

       Hrej(k,1)=H; %number of host eggs surviving at the end of T days?
       Prej(k,1)=P; %number parasite eggs surviving at the end of T days?

       H=0; %reset number of host eggs to zero for next replicate
       P=0; %reset number of parasite eggs to zero for next replicate
      end %end loop through k replicates

     % calculate mean values for all k replicates at given p
     Hrej(b,1)=mean(Hrej); %mean number of host eggs surviving
     stdHrej(b,1)=std(Hrej); %standard deviation of host eggs surviving
     Prej(b,1)=mean(Prej); %mean number of parasite eggs surviving
     stdPrej(b,1)=std(Prej); %standard deviation of parasite eggs surviving

end %end loop through all values of p
```

(c) ***Sensitivity analysis***

A sensitivity analysis of *b*; the figure below shows H_{DIF} and P_{DIF} as a function of *b* when *p*=0.5, the probability of per day parasitism observed at our field site for chalk-browed mockingbirds parasitized by shiny cowbirds. All qualitative conclusions described in the text are robust within the ranges of *b* shown; details given below.



H_{DIF} and P_{DIF} as a function of *b,* a parameter that weighs the distribution of attacks between egg types. When *b*=1 egg types share attacks strictly according to egg numbers, *b*>1 indicates bias towards parasite eggs and 0>*b*>1 indicates bias against parasite eggs (i.e. bias towards host eggs). The probability of parasite eggs breaking when struck is lower than that for host eggs (s_H=0.9, s_P=0.3), corresponding to Figure 5a in the main text. The margin of host egg survivorship for acceptors over rejecters (H_{DIF}) increases as the bias towards parasite eggs increases, approx. ±0.2 host eggs to that expected when no bias occurs (*b*=1, indicated with a dotted line), while the margin of parasite egg survivorship has an inverse trend, decreasing as the bias towards parasite eggs increases.

Chapter 3

Two measures of success in a host's front-line defence against brood parasites

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Unpublished Manuscript

ABSTRACT

For many hosts of brood parasitic birds, their front line of defence is to mob adult parasites that approach the nest. This is commonly interpreted as an adaptation to prevent the parasite from depositing its egg. We investigated the effectiveness of mobbing by chalk-browed mockingbirds (*Mimus saturninus*) as a defence against their parasite, the shiny cowbird (*Molothrus bonariensis*), using over 350 filmed cowbird visits to nests and other direct observations. Mockingbirds only occasionally prevented cowbirds from reaching the nest, or laying once in it. More often, cowbirds breached their host's defence, aided by their agile flight, rapid laying, endurance of mobbing, and opportunistic timing, including approaching nests when mockingbirds were distracted in battle with another cowbird. Adult cowbirds present a second threat to hosts, however, in that they try to destroy host eggs prior to laying their own. Cowbirds that were mobbed while in the nest were less likely to succeed in breaking mockingbird eggs than those that were undisturbed, regardless of whether or not they laid. In this host therefore, the benefit of mobbing must be assessed by two independent measures – prevention of egg loss and prevention of parasitism. As mockingbird eggs that survive a cowbird's visit intact can go on to fledge from parasitized broods, we expect strong selection for mobbing as an anti-parasite defence in this host, even though it largely fails to prevent parasitism itself.

1. INTRODUCTION

Many hosts of brood parasitic birds defend themselves against the costs of parasitism at one or more stages of the nesting cycle. Among the most widely-documented of a host's 'front-line' defences (those that precede the act of parasitism itself) is a capacity to recognize adult parasites as a special threat and respond to them aggressively (Payne et al. 1985, McLean 1987, Briskie and Sealy 1989, Moksnes et al. 1991, Briskie et al. 1992, Mark and Stutchbury 1994, Webster 1994, Soler et al. 1999, Welbergen and Davies 2008, Fiorini et al. 2009a, Langmore et al. 2012, Trnka and Prokop 2012). We use the term mobbing to describe these aggressive responses by hosts (after Welbergen and Davies 2009), which can include chasing, striking with the beak and feet, biting, swooping, or knocking a parasite from air to ground.

As for other front-line defences, mobbing is believed to offer hosts a chance to prevent the parasite from laying in their nest (Sealy et al. 1998). While quantifying the effectiveness of mobbing has proven difficult in many host-parasite systems (Sealy et al. 1998), compelling reports tell of hosts infrequently fending off parasites from their nests (Neudorf and Sealy 1994, Budnik et al. 2001, Ellison and Sealy 2007, Kruger 2011) or even killing them (Molnár 1944, Moyer 1980). More recently, Welbergen and Davies (2009) found indirect support that mobbing by reed warblers (*Acrocephalus scirpaceus*) reduces parasitism by common cuckoos (*Cuculus canorus*), by showing that those warbler pairs that mobbed a cuckoo mount suffered less parasitism than pairs that did not mob.

For some hosts however, prevention of parasitism may not be the only, or even the principal, benefit accrued through mobbing. Adult parasites typically present two threats to a host's clutch: the addition of their own egg, which leads to misdirected parental effort, and the ruin or removal of host eggs. The cost of own egg loss to a parasitized host varies between host-parasite pairs, but may be high (Peer 2006). Where host young can be readily fledged alongside parasites, then clutch reduction can be the primary source of offspring loss in parasitized nests (Massoni and Reboreda 2002). Even for hosts whose offspring perish in the company of nestling parasites, egg loss will be worth minimizing provided there is some chance to remove the parasite in a subsequent line of defence (Robertson and Norman 1976, Sato et al. 2010). Importantly, aggressive responses shown by hosts towards parasites will not necessarily affect the incidences of clutch reduction and parasitism equally; rather, success in one measure could be independent of success in the other. Tewksbury et al. (2002) found that this applied in the context of general nest attentiveness by yellow warblers (*Setophaga petechia*) at risk of parasitism by brown-headed cowbirds. Warblers that spent more time atop the nest were less likely to have an egg removed by cowbirds, but no less likely to be parasitized. If such a distinction also held for mobbing, then selection might sometimes favour this front-line defence primarily because it prevents parasites from destroying host eggs, rather than because it prevents parasitism *per se*.

In this study, we estimate the effectiveness of mobbing by the chalk-browed mockingbird (*Mimus saturninus*) as a defence against their parasite, the shiny cowbird (*Molothrus bonariensis*), based on observations of naturally-occurring interactions. Female shiny cowbirds attack host clutches by attempting to puncture holes in eggs with their beaks, both when visiting the nest to lay, and on separate nest visits. Hosts typically then clear the nest of broken eggs. Mockingbirds are larger than their parasites (adult body masses: 70-75g vs. 45-50g respectively), a size advantage that ensures that mockingbird eggs that survive a cowbird's visit intact can be readily hatched and reared alongside cowbird nestmates in parasitized broods (Fiorini et al.

2009b, Gloag et al. 2011). Egg loss from cowbird punctures is therefore the primary cause of own offspring mortality due to parasitism for this host (Fiorini et al. 2009b). In a previous study in which we focused on the costs and benefits of a secondary host defence (rejection of parasite eggs from the clutch), we made the preliminary observation that mockingbirds commonly mobbed cowbirds in their nests, but rarely stopped them from laying, or making a puncture attack (Gloag et al. 2012). Here we investigate mobbing in detail, using an expanded dataset and new analyses to assess outcomes both in and out of the nest. Specifically, we aim to determine to what extent mobbing succeeds in preventing both clutch reduction by parasites, and parasitism itself.

2. METHODS

(a) *Study site and species*

We collected data at Reserva El Destino, Buenos Aires Province, Argentina (35°08′S, 57°23′W), during the springs and summers of 2010-2012. The site is pampas grassland, punctuated by small clusters of trees, predominantly tala (*Celtis tala*), coronillo (*Scutia buxifolia*), and molle (*Schinus longifolius*). We monitored the nesting attempts of approximately 40 chalk-browed mockingbird pairs per season. Each pair holds a territory centred upon one or more tree clusters, in which they build large open nests of sticks, lined with hair. Nesting attempts begin mid-October and continue for 10-12 weeks, with each unsuccessful attempt followed by the construction of a new nest in a new location within their territory. High rates of predation, and also abandonment due to excessive clutch reduction by cowbirds, mean that most pairs will build several nests per season. We treated nests built in the same territory within a season as having been built by the same pair. The incidence and intensity of cowbird parasitism on mockingbirds at this site is high (89% of nests are parasitized, 70% of them multiply, Gloag et al. 2012). Mockingbirds at this site have no known defences at later stages in the breeding cycle, with the exception of rejecting one cowbird egg morph laid uncommonly in their nests, the pure-white morph (de la Colina et al. 2012).

(b) *Nest filming and observations*

Most parasitism occurs within the four-day window between the appearance of the mockingbird's first egg and the onset of incubation (Fiorini et al. 2009b). During this interval, we fixed 'nest-cams' into the vegetation above nests (Handykam CCD colour microcameras), attached to digital video recorders (Lawmate PVR1000 or PVR500 ECO) and power sources concealed on the ground. Nests were filmed from 30 minutes prior to sunrise until mid-morning or, when possible, until sunset, on each day until the end of the first day of incubation (or until the nest was predated or abandoned). Nest-cams therefore captured both cowbird laying visits (defined as visits that occurred prior to sunrise) and puncturing visits (all subsequent visits). We considered a nest to be abandoned if the mockingbirds did not appear at the nest during the day's recording. An egg was scored as punctured if mockingbirds were seen to remove it following a cowbird's visit. We also checked the contents of nests each day to label new eggs, and recorded any eggs that had been broken but not removed by mockingbirds from the nest. We filmed a total of 597 days or half-days at 213 nests (2010-11: 88 nests, 41 pairs, 2011-12: 125 nests, 38 pairs).

Nest-cams captured only events at the nest. To assess therefore whether mockingbirds were able to prevent cowbirds from reaching the nest at all, we also made observations at active nests (*n*=28 nests, each of a different pair), in which nest-cams were deployed. Observation sessions took place at the time of day that cowbirds lay, beginning 45-60 mins prior to sunrise and concluding 15 mins after sunrise. We made either one session (*n*=21 nests), or two sessions on consecutive days (*n*=7 nests), per nest. The observer concealed themselves in the long grass 10-15m from the tree containing the nest, prior to first light, and noted whether a cowbird (i) approached the tree containing the nest, (ii) was mobbed by mockingbirds, and (iii) entered the tree containing the nest.

(c) ***Analysis***

Mobbing effectiveness

To determine the effectiveness of mobbing by mockingbirds in preventing parasitism, we scored the number of mobbed laying visits in which cowbirds succeeded in getting their egg in the nest, both from nest observations and nest-cam recordings. As we filmed multiple nesting attempts per mockingbird pair, and multiple days of each nesting attempt, some nests and pairs were represented more than once in the final dataset. We report results from both our complete dataset and for a subset of data that includes only the first recorded case per nest, or per pair.

We assessed the effectiveness of mobbing in preventing egg loss in three ways. First, we compared the proportion of nest visits captured by nest-cams in which a puncture attack occurred at all (defined as at least one strike of the cowbird's beak onto the clutch) when cowbirds were mobbed or not mobbed, using a Fisher's Exact Test. Second, we related the likelihood that cowbirds broke at least one egg during their visit, to the occurrence of mobbing by mockingbirds. This analysis considered those nests that contained only mockingbird eggs at the time of the puncture attack, and for which puncture success could be unambiguously assigned to a single cowbird's visit. To avoid pseudoreplication, we included only the first recorded visit per mockingbird pair within a category (mobbed or not mobbed). We used a forward stepwise logistic regression, in which our dependent variable was success in breaking an egg (yes/no) and our predictor variables were mobbing (yes/no), number of eggs present at the time of the puncture attacks (1-4), and the interaction term. Predictors were entered into the model if the resulting reduction in deviance (Δdev) was larger than the critical value of chi-square at a=0.05 and *d.f.*=1.

Finally, we assessed whether mobbing reduced the incidence of cowbird puncturing visits (i.e. non-laying visits). Preliminary nest-cam recordings indicated that mockingbirds very rarely mobbed such visits. Any mobbing or chasing of cowbirds during daylight hours must therefore occur outside of the camera's field of view. As puncturing visits can occur at any daylight hour, it was not practical for us to assess nest defence based on focal nest observations. Instead, we took advantage of nest-cam recordings made at those nests that were abandoned during the mockingbird's laying period to make an indirect estimate. We compared the number of visits made by cowbirds to active nests against those made to nests newly abandoned (i.e. on the first day of abandonment) using ANOVA. The probability of puncture attacks occurring may vary between days of the host's laying period. As almost all of the nests that were abandoned were done so on the third or fourth days of the laying period, we chose to restrict our comparison only to nests on these laying-days. If either a mockingbird's presence near the nest, or mobbing specifically, deterred cowbirds from making puncturing visits, we expected fewer such visits in active nests than in nests recently abandoned.

Cowbird behaviour

To compliment our assessment of mockingbird's defence, we scored some relevant aspects of cowbird behaviour at mockingbird nests: the time taken to lay (seconds, measured as the time between the final strike of a puncture attack and the egg being laid), the total time spent in the nest (seconds) and, when mobbing occurred, the number of blows received from mockingbirds. In addition, we noticed that when two or more cowbirds parasitized a nest on the same morning, the time interval between their arrivals was often very short (<60 seconds, hereafter referred to as 'synchronous'), such that mockingbirds were still engaged mobbing the first cowbird, or inspecting their damaged clutch, when the second one arrived. We reasoned that this could happen if cowbirds showed an opportunistic tendency to approach nests when mockingbirds were distracted. If so, then the proportion of same-day laying visits observed to be synchronous would be higher than that expected by chance. We tested this using a Monte Carlo simulation, in which x was the total number of filmed nests that were multiply parasitized on the same day, and y was the number of those nests for which the arrival interval between females was less than 60 seconds. For nests parasitized more than twice on the same morning, we considered only the intervals between the first two parasitic events. Drawing random samples from the pool of observed arrival times (relative to sunrise on the day of recording) across all filmed laying visits (n=291), we generated a frequency histogram of expected y in x if cowbird arrival times were independent, based on 100000 simulations. We considered that the observed number of synchronous cowbird arrivals was significantly greater than chance if it fell above the 95th percentile of this distribution.

We used SPSS v20.0 or MATLAB (R2010a) for all statistical tests. Errors reported are standard errors of the mean.

3. RESULTS

(a) *Does mobbing prevent parasitism?*

On approach to the nest

At all nests in which pre-dawn observations were made (*n*=28), mockingbird parents assumed conspicuous perches from first light, on high branches close to the nest (<5m). We observed a total of 26 interactions between mockingbirds and cowbirds at 17 different nests. In all cases, cowbirds would shoot rapidly towards nests from concealed positions in the long grass or nearby vegetation and one or both mockingbirds would attempt to intercept the cowbird mid-air on its approach, often prompting an aerial dogfight, in which mockingbirds pursued cowbirds as they twisted and turned attempting to reach the tree. In 22 cases, the cowbird outmanoeuvred the mockingbird and entered the tree with the mockingbird in tow (84%). Nest-cams indicate that these cowbirds were then mobbed in the nest. In the remaining 4 cases, the cowbird instead fled into nearby vegetation. Of these latter cases however, two were followed that same morning by a successful cowbird lay, which may have been the same female returning. Mobbing outside the nest therefore succeeded in preventing at most 16% of parasitism, and more likely half of that (2 of 24 attempts, where two of the 26 observed attempts are assumed to be by the same female, 8%). This proportion was similar if we considered only the first observed interaction for each mockingbird pair (2 cowbird failures out of 17 attempts, or a maximum defence success of 12%).

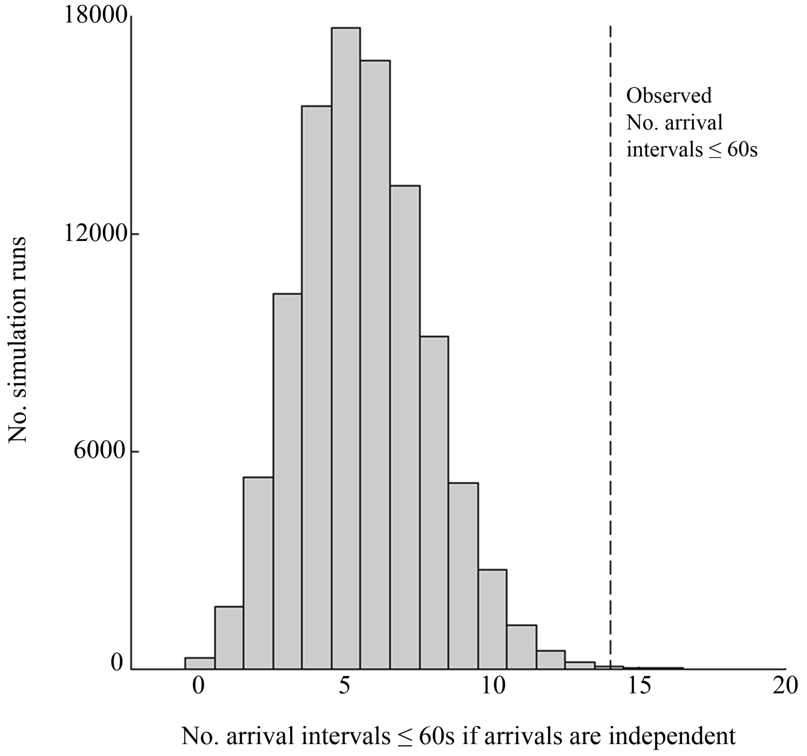

Figure 1. The frequency distribution of expected observations of cowbird arrivals at <60 second intervals, from 51 cases of same-day multiple parasitism, if cowbirds arrive independently of each other (i.e. if synchrony is generated by chance alone). The number of synchronous arrivals we observed (dotted line) was significantly greater than expected by chance. The distribution was generated from a Monte Carlo simulation with 100 000 runs, drawing with replacement random pairs of arrival times (relative to sunrise) from a pool of all the laying visit arrival times in our database (*n*=291).

On one occasion we observed a second cowbird approach the nest only seconds after a first had entered the tree in which the nest was located, pursued by both mockingbirds. Nest-cam recordings showed that such synchronous arrivals were not rare: in 14 of 51 cases of same-day parasitism filmed, the second cowbird to arrive at the nest did so within just 60 seconds or less of the previous cowbird (22%), significantly more than expected by chance if the arrival times of cowbirds were independent based on a Monte Carlo simulation, as shown in Figure 1. In most cases, second-to-arrive cowbirds were also mobbed in the nest by mockingbirds, though some escaped mobbing altogether while mockingbirds continued to attack the previous cowbird (3 of 14, 21%; see example videos ESM1-2). Synchronous arrivals sometimes resulted in two cowbirds occupying the nest at the same time (4 of 14, 29%). In such cases the cowbirds jostled with each other for space in the nest, but otherwise did not interfere with each other's laying (see example, ESM2).

At the nest

Nest-cams filmed 264 laying visits by cowbirds to active nests. Of these, in 42 cases (16%) the cowbird arrived at the nest, punctured and laid undetected, while in the remaining 222 cases the cowbird was mobbed whilst in the nest (84%). The proportion of cowbird visits that were mobbed was similar when we considered only the first laying event filmed per nest (77%, *n*=137 nests), or per pair within a season (2010: 79%, *n*=29 pairs, 2011: 73%, *n*=33 pairs). Cowbird egg-laying was rapid (6.3±0.9 seconds, *n*=153), but the time cowbirds spent in the nest was typically prolonged due to puncture attacks and host mobbing (total time spent in nest: 20.7±1.8 seconds). Mockingbirds delivered on average 17 (±2.0) blows to the head and body of the cowbird whilst it was in the nest, and in some cases upwards of 100 blows. Of all mobbed cowbirds however, just 14 of 222 were observed to flee the nest before laying an egg (see example, ESM3), and of these, almost half (*n*=6), were followed that same morning by a successful laying visit, which could have been the same female returning. Mobbing of cowbirds once they had entered the nest succeeded therefore in preventing parasitism in at most 6%, or at worst 3.6% of cases; see Figure 2. Most cowbirds that left the nest before laying an egg made puncture attacks (12 of 14). Cowbirds were sometimes trapped in vulnerable positions by mockingbirds, belly-up in the nest or nearby vegetation. While we never filmed evidence of a cowbird conspicuously injured by mobbing, at one nest that was not filmed we found a dead female cowbird impaled on a large *Coronillo* thorn, just 30cm below an active mockingbird nest and with an egg low in her oviduct. This death seems certain to be the result of being pushed onto the tree's spine by the weight of one or both attacking mockingbirds (a fatal version of the type of scenario seen in ESM 1).

(b) *Does mobbing prevent egg-puncturing?*

Cowbirds usually began striking at eggs with their beak the moment they landed in the nest, and only infrequently, when mockingbirds stood quickly and directly on the cowbird's head was puncturing prevented entirely (14 of 222 mobbed visits, 6%). This was not significantly different from the incidence of puncture attacks that preceded laying visits in which the cowbirds were not mobbed (2 of 42 visits, 5%; Fisher's Exact Test, *d.f.*=1, *p*=0.52). However, 16 of 17 cowbird visits undisturbed by mockingbirds resulted in the loss of at least one egg to puncture attack (94%), while only around half of those that were mobbed did so (12 of 25, 48%); shown in Figure 2. Based on these nests, a logistic regression model indicated that mobbing was a significant predictor of the likelihood that cowbirds succeeded in breaking at least one egg during a puncture attack (*Δdev* =11.24, *d.f*=1, *p*=0.001). Neither clutch size, nor an interaction term (clutch size*occurrence of mobbing) significantly improved the fit of this model. Notably, in all laying visits included in this analysis, the cowbird succeeded in laying an egg.

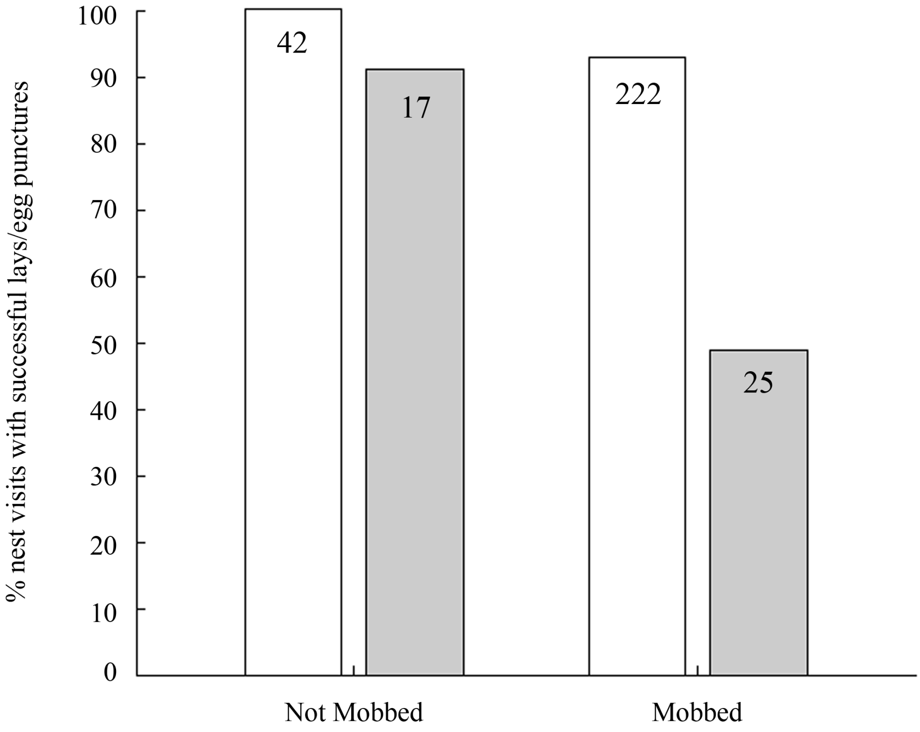

Figure 2. The proportion of visits made by shiny cowbirds to nests of chalk-browed mockingbirds in which the cowbird succeeded in laying an egg (white bars) or breaking at least one mockingbird's egg (grey bars), when they were either mobbed in the nest by mockingbirds or not mobbed. Sample sizes are given inside the bars.

We filmed 134 puncturing (non-laying) visits to active nests. Most involved a single female cowbird, though in four cases a second female was present (3%). In just 5 of all filmed puncturing visits was a mockingbird seen to arrive at the nest and chase the cowbird off-screen (4%). However, nests that were abandoned attracted significantly more puncturing visits than still active nests (abandoned: mean=1.7±0.5 *n*=15, active: mean=0.3±0.7 *n*=46, *t*=4.1, *d.f.*=59, *p*<0.001), consistent with either mobbing, or general nest vigilance, being a deterrent to cowbirds making puncturing visits. This difference was also significant when we considered only one nest per mockingbird pair (abandoned: mean=1.5±1.5, *n*=15, active: mean=0.26±0.5, *n*=46, *t*=3.7, *d.f.*=32, *p*=0.001).

4. DISCUSSION

Direct and recorded observations of chalk-browed mockingbirds mobbing their parasite, the shiny cowbird, revealed that this front-line defence can both prevent cowbirds from laying, and prevent the mockingbird's eggs being broken during cowbird attacks, and that these outcomes are often independent of each other.

Mockingbirds' success in preventing cowbirds from accessing the nest and laying in it was limited. In flight, cowbirds were quicker and more agile than mockingbirds, so usually triumphed in aerial chases on approach to the nest. Cowbirds also appeared to take advantage of moments when their hosts were distracted to approach nests: thus we found that where two cowbirds parasitized a nest on the same day, the second was far more likely to arrive in close succession with the former (<60 second afterwards) than expected by chance alone. Once in the nest, cowbirds were then able to lay, not only very rapidly (as characterizes all parasitic birds, Davies 2000), but whilst enduring a barrage of blows to the head, eyes and body from their much larger hosts. Very few cowbirds that reached the nest left it without laying an egg, regardless of the mockingbird's efforts.

In mockingbirds therefore, selection may favour a vigorous mobbing defence as much or more because it reduces the probability of losing host eggs, than because it prevents the parasite laying. While mobbing cowbirds in the nest rarely prevented puncture attacks occurring altogether, it did reduce by almost half a cowbird's success in breaking one or more eggs, compared to cowbirds that were left to puncture eggs undisturbed. It is possible that mobbing also reduces the incidence of post-sunrise puncturing visits (i.e. non-laying visits) made by cowbirds, although this is less clear from our data. On the one hand, we filmed very few such visits that were interrupted by mockingbirds, suggesting that cowbirds regularly access nests undetected during daylight hours. However, we did find that recently abandoned nests attracted more puncturing visits than active nests, suggesting that the incidence of puncturing visits would be even higher were mockingbirds not present to defend.

What are the implications of a front-line defence against clutch reduction? The selective pressures on the various potential lines of host defences are likely interdependent (Britton et al. 2007, Kilner and Langmore 2011). For example, a host that can reliably detect and reject foreign eggs from the nest may experience relaxed selection for the ability to do likewise with foreign nestlings (Kilner and Langmore 2011). The role of mobbing in reducing host egg loss may too have knock-on effects for the evolution of later lines of defence. In the case of mockingbirds, mobbing could, firstly, reduce the selective pressure on subsequent defences against parasitism by decreasing the time and effort mockingbirds can expect to waste on rearing parasite young. The more mockingbird eggs that survive to hatch, the greater the likelihood that their cowbird nestmates, as the smaller chicks, will starve early in the nesting period; indeed, Fiorini et al (2009b) found that, in parasitized nests where all four mockingbird eggs survived intact and hatched, cowbird nestlings always perished a few days after hatching. A second possibility is that mobbing may amplify the benefit of a ‘dilution effect’ in multiply parasitized clutches, which may in turn disfavour the evolution of parasite egg rejection. Gloag et al. (2012) found that the presence of cowbird eggs in a clutch reduced the risk of mockingbird egg loss during subsequent parasite attacks. In the present study, we considered only the effects of mobbing for clutches that contained solely mockingbird eggs, but it may be that the combined effects of mobbing, and the presence of previously-laid parasite eggs in the nest, leads to greater survivorship of mockingbird eggs that either factor on its own.

Mockingbirds are unlikely to be the only hosts for which mobbing has two measures of success. Clutch reduction behaviours differ, however, between parasitic species and can not always be separated from the act of parasitism itself. The broken eggs in the nests of hosts of great spotted cuckoos (*Clamator glandarius*) for example, result in part from puncture attacks, but also in part from the parasite’s robust egg falling onto them and cracking them when it is laid (Soler and Martinez 2000). Other parasites, such as common cuckoos, remove host eggs during laying visits rather than puncture them (Davies and Brooke 1988), and it remains to be investigated whether harassment from hosts can ever lead these parasites to bypass egg-removal when laying, or to remove fewer eggs than they otherwise might. Also, not all hosts will benefit from preventing clutch reduction independently of parasitism. If host eggs that hatch are certain to die later due to the presence of the parasitic chick, there will, of course, be little value in a front-line defence that curbs clutch reduction but allows egg laying. Nevertheless, we propose that further investigation of the role of mobbing in preventing host egg loss, as well as parasite egg gain, will reinforce the idea that front-line defences against parasites are not always solely defences against the act of parasitism itself.

5. ACKNOWLEDGEMENTS

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Chapter 4

Tremulous begging calls of a generalist brood parasite exploit common avian provisioning rules

Gloag, R. & Kacelnik, A.
Unpublished Manuscript

ABSTRACT

The nestlings of some brood parasitic birds mimic the acoustic structure of their host's begging call, presumably so as to best secure host parents' care. Other parasites, however, produce begging calls entirely unlike those of their host's young. We show that in these cases, the parasite's call structure can still act in its favour. Nestlings of the generalist brood parasitic shiny cowbird (*Molothrus bonariensis*) produce long 'tremulous' calls comprised of repeated syllables, readily distinguishable from the short 'chip' calls of a common host, the house wren (*Troglodytes aedon*). We broadcast both call types, matched for call rate and amplitude, at unparasitized wren broods and found that parents provisioned more during broadcast of cowbird calls than during those of their own species' young. Further experiments indicated that in this case (i) the tremulous call structure functions analogously to a rapid call rate: thus the broadcast of clusters of multiple wren calls provoked provisioning rates similar to that of cowbird calls, while the broadcast of single cowbird call syllables provoked provisioning rates similar to that of wren calls, and (ii) aptly for a generalist, the effect of cowbird call structure is not host-specific; when both experiments were repeated in a 'parasite-naïve' passerine (the great tit, *Parus major*), parents' responses closely mirrored those of house wrens. Our results suggest that the shiny cowbird's begging calls exploit provisioning responses widespread among birds, and reveal an alternative route by which the acoustic structure of parasite calls may be tailored to optimize host care.

1. INTRODUCTION

For young brood parasitic birds, survival depends on the receipt of care and resources from other species, their hosts. While the simple presence of the parasite in the nest may ensure it receives some degree of care, parasites also benefit from producing begging signals that provoke appropriate provisioning responses from their adopted parents. Indeed in some cases, in order to meet its needs, a parasite's begging must be not just an effective stimulus of parental care, but a superior stimulus to that of the host's own young (Davies et al. 1998).

The vocal component of the begging signal is widely recognised to play a key role in such host manipulation for many parasites, on the basis that they vocalize more loudly and more rapidly than do host young (Woodward 1983, Gochfeld 1978, Davies et al. 1998, Dearborn and Lichtenstein 2002, Redondo and Zuniga 2002). In doing so, parasites exploit the sensitivity of avian parents to these call parameters, which in unparasitized broods are reliable signals of brood need (Godfray 1991, Rodríguez-Gironés et al. 1996, Kilner et al. 1999, McGrath et al. 2010). The common cuckoo (*Cuculus canorus*) for example, calls at rates equivalent to a whole brood of the young of a common host, the reed warbler (*Acrocephalus scirpaceus*), and is rewarded accordingly (Davies et al. 1998).

If and how avian parents respond to the acoustic structure of the begging call itself however, and whether such responses are exploited by parasites, remains poorly understood. Nestling begging call structure varies greatly among species, even between closely related taxa (Marler 2002). One commonly proposed route therefore by which a parasite's call structure could best secure its host's care is to mimic the calls of the host's young. Mimicry would presumably be favoured wherever hosts discriminate in favour of their own-species' call type, which they may do either as a means to detect and reject parasite young (Langmore et al. 2003), or due to some pre-existing sensory bias (Ryan 1990). Consistent with this view, many parasites have begging calls that closely resemble those of their host's young (Reed 1968, Mundy 1973, McLean and Waas 1987, Redondo and Arias de Reyna 1988, Redondo 1993, Fraga 1998, Langmore et al. 2008, De Mársico et al. 2012), including some generalist parasites that produce different call types in different hosts (Reed 1968, McLean and Waas 1987, Redondo and Arias de Reyna 1988, Langmore et al. 2008).

There exists however a second possible, but untested, route by which call structure could be employed to a parasite's advantage. Rather than mimic host vocalizations, a parasite's call might exaggerate salient acoustic features that in host young indicate brood need, in the same way as the rate and volume of calls can be exaggerated. In this case, we assume that the host shows no especial bias towards its own species' overall call structure. Instead, call-for-call, the parasite's vocalizations will provoke the greater provisioning effort. The idea that parasites might produce call types more effective than those of host chicks was proposed by Madden and Davies (2006), who suggested it could explain why calls of the generalist common cuckoo (*Cuculus canorus*) vary when reared in one or the other of two common hosts, but do not resemble the host's calls in either case. Importantly however, it is not necessary that a 'non-mimetic' call structure be host-specific in order to be effective. Where a parasite's calls enhance host investment via differences, rather than similarities, to those of the host young, they could do so for a whole range of host species, provided that the acoustic features they exaggerate are relevant across hosts.

Here, we study the role of the begging call structure of the generalist brood parasitic shiny cowbird (*Molothrus bonariensis*) in manipulating host care. Shiny cowbirds use a large and diverse set of hosts throughout South and Central America, and are commonly reared alongside some host nestlings (Ortega 1998). A previous qualitative report suggests that this parasite's begging calls are neither mimetic nor polymorphic across common hosts (Fraga 1998); rather, nestlings produce a distinctive 'tremulous' call.

We first characterized and compared shiny cowbird and host nestling call structure in naturally parasitized nests of a common host, the house wren (*Troglodytes aedon*). We then performed a series of playback experiments with unparasitized broods to test: (i) the effectiveness of cowbird begging calls in stimulating provisioning relative to house wren calls, independent of call rate and volume, (ii) the acoustic properties responsible for variation in parent's provisioning responses between call types, and (iii) whether these acoustic properties are species-specific in their effect. For the latter, we repeated our house wren experiments in a 'parasite-naïve' passerine, the Eurasian great tit (*Parus major*). As great tits share no coevolutionary history with cowbirds, nor are they close relatives of any extant cowbird hosts, we considered that a strong provisioning response to cowbird calls by this species would indicate that the parasite's call structure exploits a widespread feature of parent-offspring communication in birds.

2. METHODS

(a) *Comparison of call structures from field recordings*

To the human ear, the vocalizations of nestling cowbirds differ greatly from those of their wren nestmates. We characterized quantitatively these differences, comparing the acoustic structure of the calls of nestlings of each species. We made audio recordings at naturally parasitized wren nests, built in nestboxes, at our fieldsite Reserva El Destino, Buenos Aires Province, Argentina (35°08′S, 57°23′W), during the springs and summers of 2008-2010. At this site, wrens lay a clutch of 4-6 eggs and parasitized nestboxes typically receive one or two cowbird eggs (Tuero et al. 2007). We checked nests daily in the period prior to the onset of incubation and, following the appearance of a cowbird egg, we standardized clutch compositions so that on hatching each nest contained one cowbird and 3-4 same-age wren young (see Gloag et al. 2011 for details of clutch manipulations). We made one-hour *in situ* audio recordings of nestling begging during mornings when broods were 4,6,8 and 10 days post-hatch (cowbird fledging age: 12-14 days, Kattan 1996) using a lapel microphone at the lip of the nest cup, attached to a digital audio recorder (M-Audio Microtrack; 24-bit, 96 kHz). Of an initial 16 nests recorded with 4 day-old nestlings, predation reduced the sample to 9 nests by day 10. Recordings were uploaded into RavenPro 1.4 (Cornell Lab of Ornithology, NY) and displayed as spectrograms using default settings (filter bandwidth: 248 Hz, frequency grid spacing: 172 Hz, time grid resolution: 2.9 ms). We scored the first five clearly defined calls of each species (those with no overlapping calls) from each of three begging bouts and took mean values of five variables, ignoring harmonics: maximum and minimum frequency (Hz), frequency bandwidth (Hz), peak frequency (Hz, the frequency for which amplitude is greatest) and call duration (seconds). We made multivariate comparisons of call structures using MANOVAs at each age, followed by post-hoc pairwise comparisons (paired t-tests with sequential Bonferroni adjustments on α levels, Holm 1979).

From these recordings, we also calculated and compared mean call rates of both species. Cowbird call rate was estimated by counting from spectrograms the number of calls made per begging bout, in each of three begging bouts, and taking the mean value (calls per second). Wren call rate was estimated using the same procedure, but counting all wren calls and then dividing by the total number of wren chicks in the nest. We used paired t-tests to compare call rates of cowbirds and their host nestmates at all ages. These and other statistical tests in this study were performed in SPSS (v.20). All errors given are standard errors of the means.

Cowbirds are not known to show host-specific call polymorphisms (Broughton et al. 1987, Fraga 1998), but see Suppl. Material for a comparison of cowbird calls between common hosts at our study site.

(b) *Playback Experiments*

To assess if and how shiny cowbird nestlings benefit from the acoustic structure of their begging call, independent of the rate or amplitude of calling, we performed a series of playback experiments in three stages. The broadcast of supplementary calls at a nest is known to prompt at least temporary increases in provisioning effort in many species and has been widely used to investigate the effect of the vocal component of the brood begging signal on parental provisioning (Hinde and Kilner 2007). Based on our field recordings, we chose to perform playback experiments at 6 days post-hatch, an age approximately midway in the rapid-growth phase for nestling cowbirds (Kattan 1996) and at which the cowbird's distinctive tremulous call is already well developed. Each experiment was performed in one of four breeding seasons, between 2009-2011.

(i) *Cowbirds calls vs. house wren calls*

We first compared the provisioning effort of house wrens to nests in which their brood's begging was supplemented by the broadcast of either a cowbird's begging calls or those of an additional house wren. We generated ten unique call samples of 6-day-old cowbirds and 6-day-old house wrens by selecting between 15-20 calls per species, per nest, from spectrograms of field recordings from 10 parasitized nests. These were used to create 10 second playback samples, each with a fixed call rate (1.8±0.1 calls per second, which was the mean rate across species observed in field recordings, see Results) and with RMS (root mean square) amplitude standardized within and between samples; examples are given in Figure 1. We identified nestboxes at our fieldsite with unparasitized house wren nests of between 4-6 chicks (modal brood size: 5 chicks, Tuero et al. 2007). When chicks were 5 days post-hatch, nestboxes were fitted with a microcamera (Handykam) and lapel microphone (Sony), run by cables to a hide 10m away from the nest and connected to a digital video recorder (JXD990) and digital audio recorder (96Hz, M-Audio MicroTrack) respectively. A keychain microspeaker (2cm^3, Purelygadgets Ltd) was embedded in the material of the nest wall, connected to an audio player in the hide (Apple iPod), allowing playback into the nest cup. After installation, nests were left for 24 hours to give parents time to adjust to the disturbance. Playback experiments were conducted the following mornings (7am-12pm) when chicks were 6 days post-hatch. Nest interiors were viewed in real time from the hide, with playback initiated each time a parent entered with food, and paused when the parent exited or began to incubate. Each wren nest (*n*=20) received three 60 minute sessions: (i) broadcast of cowbird begging calls, (ii) broadcast of house wren begging calls, and (iii) no broadcast (control). The control treatment allowed us to confirm that any differences between the broadcast treatments represented respective increases from baseline provisioning rates. Each session was preceded by a 20 minute interval of no broadcast, and session orders were rotated between nests (each permutation used 3 or 4 times).

We scored the number of feeding visits made to the nest by parents in each session. When possible, we also estimated the size of prey delivered, to determine whether parents adjusted their foraging effort via prey selection (% bill width, or %BW), although in some nests prey size could not be accurately assessed due to the position of the camera. We then tested for an effect of playback on provisioning rate and prey size via repeated measures ANOVAs, followed by post-hoc pairwise comparisons with α levels adjusted within each experiment (sequential Bonferroni method). If the begging calls of nestling cowbirds are better stimulants of house wren parental care, call for call, than those of nestling house wrens, we expected their broadcast would provoke a greater relative increase in food delivered to the nest.

(ii) *Tremulous calls vs. monosyllabic calls*

Our second playback experiment aimed to tease apart which differences between the acoustic structures of shiny cowbird calls and house wren calls were principally responsible for any differences in provisioning effort they elicited. We considered call structure differences to fall into two broad categories, either or both of which might drive differences in parent's responses (Leonard and Horn 2001): one, the number of syllables in a call and/or its duration, and two, call frequency and/or bandwidth. We presented nests with playback samples of natural wren and cowbird calls (as used in part (i)) and also with two novel call types, generated in RavenPro by editing our existing playback samples: a 'truncated-cowbird' call sample, in which each sample-call was just one syllable of a natural cowbird call, and a 'clustered-wren' call in which 4 to 6 wren calls were compressed together to create an artificial tremulous structure; details are given in Figure 1. These experimental call types altered syllable number and call duration (the latter necessarily increasing with the former) but otherwise retained the given species' acoustic properties (e.g. max and min frequencies, bandwidth).

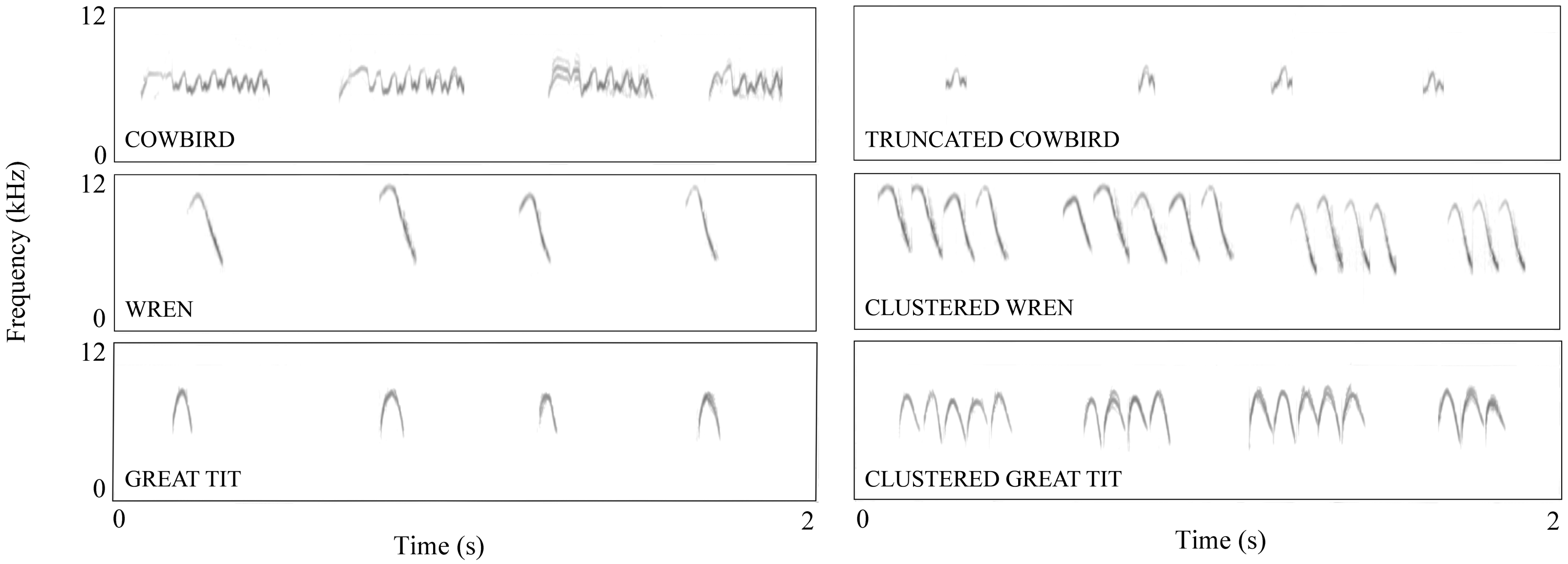

Figure 1. Example calls from samples of 6 day-old shiny cowbirds, house wrens (a common host) and great tits (a non-host), as used in playback experiments: both natural call types (left) and modified call types, in which syllable number and call duration was manipulated (right). All samples were broadcast at the same call rate and RMS amplitude.

Experimental procedure and data analysis followed that described above for the previous playback experiment, except that here each house wren nest (*n*=24) received four 40-min broadcast sessions of: (i) cowbird begging calls, (ii) wren begging calls, (iii) truncated-cowbird begging calls, and (iv) clustered-wren begging calls. Sessions were preceded by a 20min interval of no broadcast, and session orders were rotated between nests (Latin square design, each permutation used 6 times). This broadcast regime generated four planned comparisons of interest in two reciprocal sets: if parent's responses were sensitive to a call's duration and/or syllable number, then we expected differences in provisioning effort during broadcast of calls of the same species origin but different syllable number (i.e. cowbird call vs. truncated-cowbird calls, and wren calls vs. clustered-wren calls). If parent's responses were sensitive to the frequency parameters of calls, we expected differences in responses to broadcast sessions of the same syllable number, but different species origin (truncated-cowbird calls vs. wren calls, and cowbird calls vs. clustered-wren calls).

(iii) *Responses in a parasite-naïve species*

Finally, we aimed to assess whether the relative response to the shiny cowbird's tremulous call was specific to house wrens, or consistent with general avian provisioning habits, by repeating both previous experiments (parts (i) and (ii) above) in a parasite-naïve species, the great tit (*Parus major*). Great tits are cavity-nesting passerines (adult masses c.18g), allopatric with shiny cowbirds and with no known history of interspecific parasitism. Like house wrens, the begging calls of 6 day-old great tit nestlings are monosyllabic and, to the human ear, unlike those of same-age cowbirds; see Figure 1. If the effectiveness of cowbird calls in provoking parental provisioning stemmed from exploitation of a shared provisioning rule, then broadcast treatments elicited in great tits should be qualitatively similar to those of house wrens. Experiments were conducted on a population nesting in boxes at Wytham Woods, Oxfordshire, England (51°46′N, 1°20′W). We used the same cowbird call samples as for our experiments in house wrens, and generated great tit call samples from recordings made at 10 nests with 6-day old chicks, in the same manner as we did for house wrens, with all samples matched for call rate and RMS amplitude; see Figure 1. We identified nests with brood sizes between 8-11 chicks (modal brood size: 8 chicks). All playback procedures followed those described for house wrens, for Experiment 1 (equivalent of part (i) above, *n*=18) and Experiment 2 (equivalent of part (ii) above, *n*=20), with the exception that during Experiment 1, due to high synchrony of available nests this year, experiments were performed either in the morning (7-12am, *n*=12) or in the afternoon (12.30-5pm, *n*=6), with treatment orders equally represented between morning and afternoon slots.

3. RESULTS

(a) *Comparison of call structures from field recordings*

Cowbird call structures differed significantly from those of same-age wren nestlings at all ages (MANOVAs; day 4: *F*=820.4, *p*<0.001, day 6: *F*=479.7, *p*<0.001, day 8: *F*=435.6, *p*<0.001, day 10: *F*=726.5, *p*<0.001). These differences were evident in all five acoustic variables assessed, with mean values of cowbird calls being lower in both maximum and minimum frequency and longer than those of the wren chicks sharing their nest (p<0.05 in all cases), as well as generally narrower in bandwidth (p<0.05 at all ages, except at 10 days post-hatch when no significant difference between species was detected), and lower in peak frequency during the first half of the nesting period ($p<0.05$ at 4 and 6 days of age, though no significant difference between species was detected at 8 and 10 days of age). The values of these variables and of test statistics are given in Suppl. Material. Spectrograms of example calls are shown in Figure 2, and video of an example begging bout is given in ESM1.

Cowbird nestlings produced their calls at similar rates to those of same-age wren nestlings sharing their nest throughout most of the nestling period (day 4: cowbird 1.5±0.7 calls/second, wren 1.5±0.5 calls/second, t=-0.29, *d.f.*=15, p=0.76, day 6: cowbird 1.7±1 calls/second, wren 1.9±0.9 calls/second, t=-2.03, *d.f.*=13, p=0.06, day 8: cowbird 1.8±0.2 calls/second, wren 2.2±0.2 calls/second, t=-1.8, *d.f.*=9, p=0.094), with wrens calling at marginally significantly higher rates than their cowbird nestmates by 10 days post-hatch (cowbird 1.7±0.08 calls/second, wren 2.1±0.2 calls/second, t=-2.3, *d.f.*=8, p=0.05).

(b) *Playback experiments*

(i) *Cowbird calls vs. house wren calls*

Broadcasts at the nest had a significant effect on the provisioning rates of house wrens ($F_{2,18}$=5.19, p=0.017). Differences were in the cowbird's favour, with parents making more feeding visits to their broods during broadcast of cowbird begging calls than during either broadcast of house wren begging calls (p=0.013), or a control period of no broadcast (p=0.005); Figure 3a. During broadcast of house wren calls, provisioning rates were higher than when no broadcast occurred, but not significantly so (p=0.26). No difference in the size of prey delivered between broadcast sessions was detected (cowbird: 296±84%BW, wren: 265±72%BW, no broadcast: 252±54%BW, $F_{2,9}$=2.33, p=0.15).

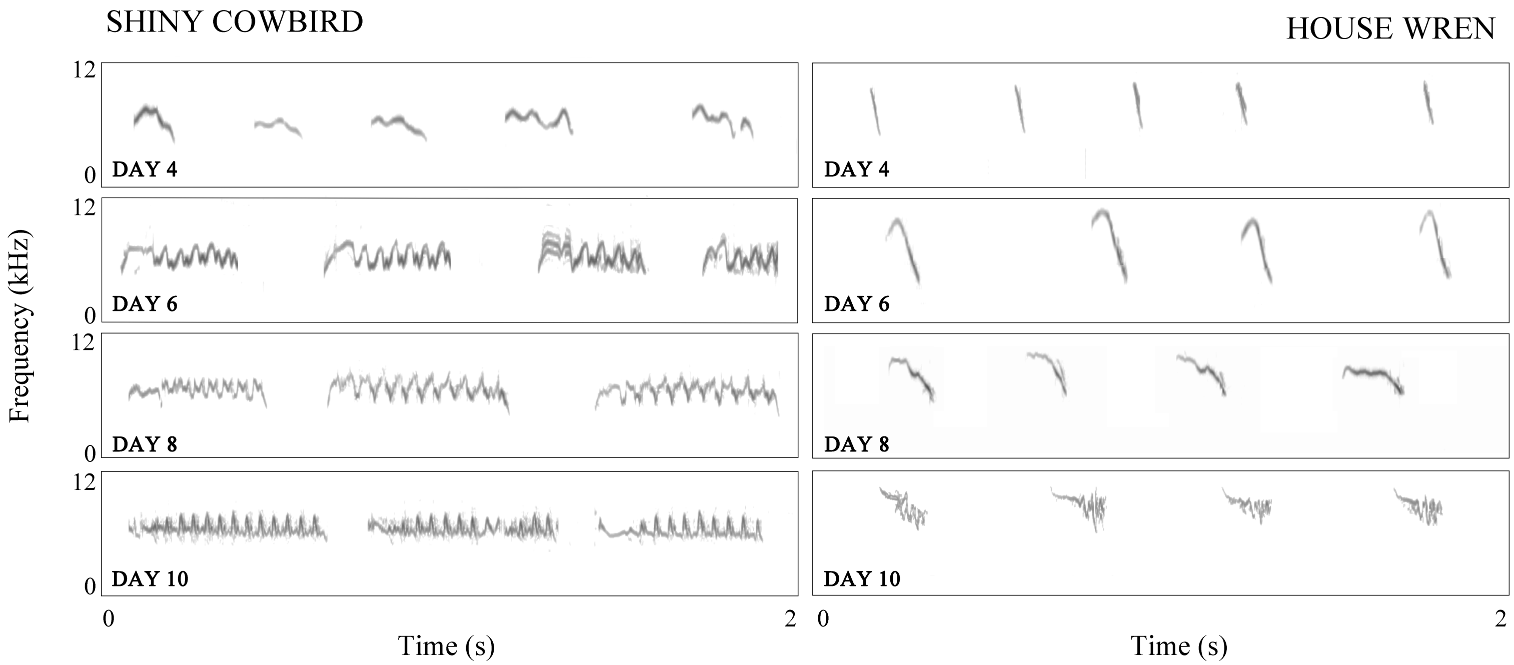

Figure 2. Example begging calls of a nestling shiny cowbird (left) and nestling house wren (right), taken from field recordings at a naturally parasitized house wren nest. Cowbird and house wren calls differ significantly in acoustic structure at all ages.

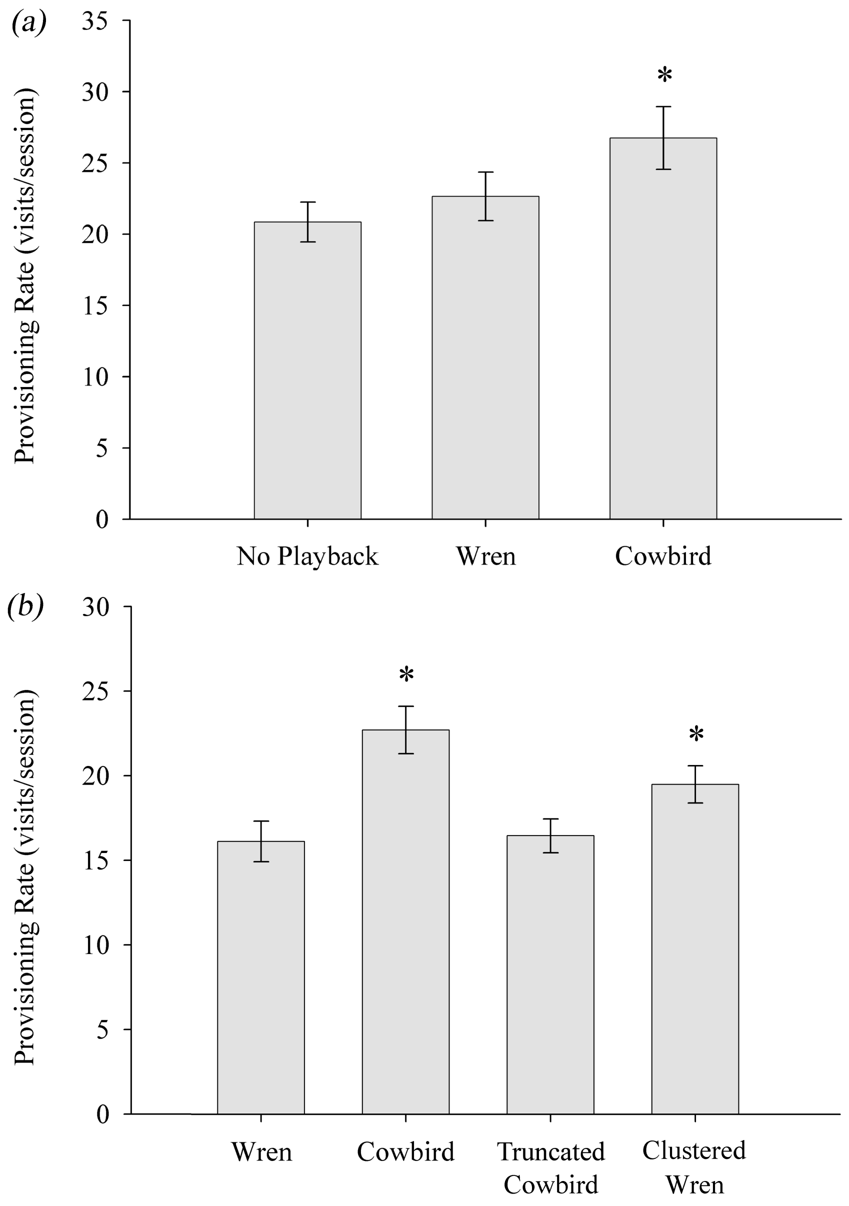

Figure 3. Mean provisioning rates (± s.e.) of a common host, the house wren, to their broods during broadcast sessions of (a) one of three treatments: cowbird begging calls, wren begging calls, and no broadcast (*n*=20), and (b) one of four treatments: cowbird begging calls, wren begging calls, truncated-cowbird calls (single syllables of a cowbird call) and clustered-wren calls (clusters of end-to-end wren calls) (*n*=24). Treatments marked with an asterisk are significantly different to those without an asterisk.

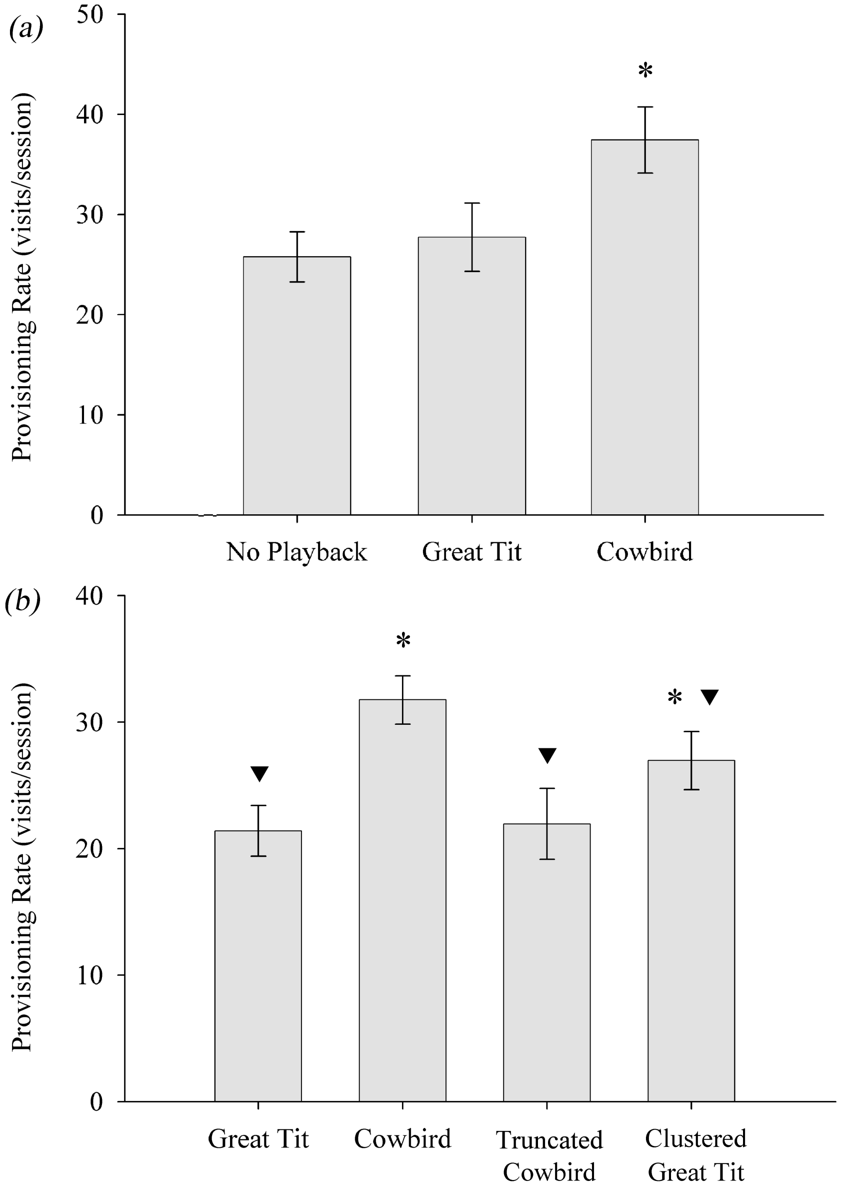

Figure 4. Mean provisioning rates (± s.e.) of a parasite-naïve bird, the great tit, to their broods during broadcast sessions of (a) one of three treatments: cowbird begging calls, great tit begging calls, and no broadcast (*n*=18), and (b) one of four treatments: cowbird begging calls, great tit begging calls, truncated-cowbird calls (a single syllable of a cowbird call) and clustered-great-tit calls (several calls end-to-end) (*n*=20). Treatments marked with an asterisk are significantly different to those without an asterisk, and treatments marked with a triangle are significantly different to those without a triangle.

(ii) *Tremulous calls vs. monosyllabic calls*

When house wrens were presented with both natural call types and those modified to alter their syllable number and duration, broadcast session again had a significant effect on provisioning rates ($F_{3,21}$=7.81, *p*=0.001), but not prey sizes (cowbird: 273±121%BW, wren: 247±82%BW, truncated-cowbird: 249±79%BW, clustered-wren: 252±86%BW, $F_{3,16}$=0.5, *p*=0.69). Here, differences were consistent with syllable number and/or call duration as being the acoustic features critical to a cowbird call's effectiveness; as shown in Figure 3b. Tremulous calls provoked significantly higher provisioning rates than their monosyllabic counterparts, both when call types were of cowbird origin (cowbird vs. truncated-cowbird, *p*<0.001) and of house wren origin (wren vs. clustered-wren, *p*=0.01). We did not detect significant differences in parent's responses to calls on the basis of their frequency and/or bandwidth; that is, during broadcast of truncated-cowbird calls, provisioning rates were not significantly different to those during broadcast of natural wren calls (*p*=0.75), and reciprocally, provisioning rates during broadcast of natural cowbird calls were not significantly different from those during clustered-wren call playback (*p*=0.06).

(iii) Responses in a parasite-naïve species

Responses to broadcasts by great tits closely mirrored those observed in house wrens. In Experiment 1, omnibus tests indicated a significant effect of broadcast session on provisioning ($F_{2,16}$=15.39, *p*<0.001) but not on the size of prey delivered (cowbird: 203±50%BW, great tit: 173±37%BW, no broadcast: 209±58%BW, $F_{2,12}$=3.13, *p*=0.08). Great tit parents made significantly more provisioning visits to nests during broadcast of cowbird calls than during broadcast of either their own species' begging calls (*p*=0.001) or a control period in which no broadcast occurred (*p*<0.001). During playback of great tits calls, provisioning rates did not increase significantly above the no-broadcast baseline (*p*=0.49); Figure 4a.

In Experiment 2, omnibus tests likewise showed that broadcast session had a significant effect on provisioning rates ($F_{3,17}$=11.41, *p*<0.001), but not prey size (cowbird: 196±43%BW, great tit: 199±51%BW, truncated-cowbird: 206±55%BW, clustered-great tit: 235±70%BW, $F_{3,11}$=3.39, *p*=0.06). As for house wrens, a call's syllable number and/or duration were the principal acoustic parameters generating differential responses in parent's provisioning effort. Thus while we found no significant differences in provisioning rates when comparing call types that had an equivalent number of syllables (i.e. truncated-cowbird calls vs. great tit calls, *p*<0.84; and, cowbird calls vs. clustered-great tit broadcasts, *p*<0.044, α=0.025), great tits did make significantly more feeding visits during broadcasts of cowbird calls than broadcasts of truncated-cowbird calls (*p*<0.001). In the counterpart to this latter comparison, between great tit calls and clustered-great-tit calls, we found the same trend, although the difference in this case fell short of significance by *a priori* adjusted α levels (*p*<0.047, α=0.016); Figure 4b.

4. DISCUSSION

A host preference for parasite call type

The acoustic structure of a brood parasite's begging call has previously been proposed to secure the best host care by closely matching that of the host's young (Mundy 1973, Redondo 1993, Langmore et al. 2008, De Mársico et al. 2012). We found, however, that shiny cowbirds produce begging calls markedly different in acoustic structure to those of their house wren host, and that this difference acts in the cowbird's favour. When we broadcast the call types of both species, matched for call rate and amplitude, at unparasitized broods, wrens provisioned on average 20% more in response to cowbird call broadcast than that of their own species' calls.

Why would hosts show a greater relative provisioning response to a parasite's call? If, as a starting point, we assume that house wrens' behaviour is adaptive when tending to their own young, then cowbird calls must exaggerate call features that typically indicate brood need. Young shiny cowbirds vocalize at similar rates to those of their same-age house wren nestmates, but each cowbird call is longer than a house wren call, being comprised of multiple, repeated syllables. We found that the broadcast of cowbird calls provoked a similar provisioning effort from house wrens as the broadcast of clusters of multiple wren calls, and that, in both cases, the broadcast of these long, tremulous call types provoked greater provisioning rates than their short, monosyllabic counterparts (i.e. single house wren calls, or single syllables of cowbird calls). Thus each syllable of a cowbird's call makes a contribution to the brood's begging signal equivalent to that of a single wren chick's call. Whether the repetition of syllables in the cowbird's call structure is integral to its effectiveness however is uncertain. Possibly, wrens simply respond to the net sound energy produced by the brood, irrespective of its acoustic properties, in which case even a long monotone whistle would provoke a higher provisioning effort than a young wren's call. In either case, in the absence of parasitism, house wrens would be able to reliably adjust their provisioning to brood need, given that both net syllable rate and net sound energy will increase as brood size, brood age and/or brood hunger increase (Rodríguez-Gironés et al. 1996, Magrath et al. 2010). When parasitized however, the same rule-of-thumb leads wrens to work harder in response to a cowbird's begging calls than in response to those of their own young. The long tremulous nature of the shiny cowbird's call therefore manipulates house wren behaviour in a manner similar to the exaggerated call rates employed by another brood parasite, the common cuckoo (Davies et al. 1998, Kilner et al. 1999).

Any further acoustic differences between wren and cowbird calls must play at best minor roles in generating differences in provisioning behaviour, as we failed to detect differences in parent's responses during broadcasts of calls that differed in species origin, but not duration or syllable number. Perhaps call duration simply trumps all other structural features in determining parent's responses, with longer calls always provoking the higher provisioning effort. The work of Madden and Davies (2006) shows that this is not the case, however, for the reed warbler (*Acrocephalus scirpaceus*) which, when broadcast two types of common cuckoo calls, favoured the shorter of the two. Host sensitivity to the frequency parameters of begging calls might then depend on a diverse range of factors, with respect to both host ecology (e.g. habitat type, Briskie et al. 1999, Leonard and Horn 2005) and life history (e.g. the incidence of extra-pair paternity, Madden and Davies 2006). In addition, unlike the hosts of evictor cuckoos, cowbird hosts will not generally be faced with a wholly foreign begging signal when attending parasitized broods, but rather the combined signal of parasite and own young, and this may be an important distinction. In the present study, we focused only on the provisioning outcomes provoked by call broadcasts, rather than how they were mediated, and it remains to be investigated to what extent, if any, the presence of the house wren's own offspring influence their responses to cowbird calls. Possibly, the effects we observed are generated only by a combination of cowbird and wren vocalizations (i.e. the vocal signal of a mixed brood may be more than the sum of its parts). Alternatively, nestling wrens may adjust their behaviours when exposed to a cowbird's calls, and so affect parent's responses.

An apt call structure for a generalist parasite

To increase provisioning when confronted with an increase in the brood's call rate (or net sound production) is likely a widespread provisioning rule among avian parents that rear altricial young (Hinde and Kilner 2007). Thus we expect that the high relative provisioning response provoked by a cowbird's tremulous call will not be unique to a particular host, such the house wren. In support of this, we found that when we replicated our playback experiments in the great tit, a species that has no coevolutionary history with South American parasites, parents also provisioned more in response to the broadcast of cowbird calls than to that of their own species' chicks. Furthermore, as for house wrens, we found that great tits' responses to the broadcast of modified call types were consistent with syllable repetition and/or long relative duration as the principle features making cowbird calls the better stimulants. Clearly cowbird calls did not evolve specifically to exploit this allopatric species. That they are able to do so thus indicates, at the very least, that a cowbird call's effectiveness in house wrens is not a product of a host-specific adaptation on the cowbird's part.

Most likely, a long tremulous begging call is an apt call type for an extreme generalist parasite such as the shiny cowbird, as it is will provoke greater provisioning rates than those of a host's young in any host whose own calls are relatively short. This could be equally said of course for any generalizable begging adaptation, such as calling especially loudly or rapidly (Davies et al. 1998, Dearborn and Lichtenstein 2002), or begging in response to a wide variety of stimuli (Hauber 2003). Nevertheless, it is interesting that of the two other cowbird species for which the begging call structure has been documented, the specialist screaming cowbird (*M. rufoaxillaris*) produces calls that closely resemble those of its primary host (Fraga 1998, De Mársico et al. 2012), while the brown-headed cowbird (*M. ater*), which parasitizes upwards of 200 hosts (Lowther 1993), produces a long tremulous call similar to that of shiny cowbirds (Broughton et al. 1987). Presently, there is no evidence to suggest that long or tremulous call structures are especially rare amongst birds generally, or Icterids in particular (Lorenzana and Sealy 1996). It thus remains to be established whether a shiny cowbird's tremulous call structure has evolved as a consequence of their parasitic habits, or was a 'preadaptation' present in their non-parasitic ancestors, and from which they have subsequently benefited.

We suggest there are at least two routes therefore by which the acoustic structure of a brood parasite's begging call facilitates host care: mimicry of host young's calls (Langmore et al. 2003), or exaggeration of acoustic features that are used by hosts to gauge the needs of their young. In shiny cowbirds, we find the first example of the latter. In their case, the call's syllable number and duration are exaggerated relative to those of their house wren host, and likely additional hosts too. In principle, however, a similar benefit could be achieved via any acoustic feature of a parasite's call that exploits the provisioning rules of their host of hosts. It remains for future studies to reveal whether the begging call structures of other brood parasites that lack vocal mimicry may similarly have a role to play in manipulating host care.

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7. SUPPLEMENTARY MATERIAL

(a) *Test for host-specific polymorphisms in nestling cowbird calls*

Based on qualitative comparisons of the calls of nestling shiny cowbirds recorded and observed in various hosts in Argentina, Fraga (1998) suggested that shiny cowbird call structure does not differ according to the host in which they are reared. This is in line with evidence from nestling brown-headed cowbirds, the calls of which Broughton et al. (1987) found did not differ with host. The presence of host-specific call polymorphisms in shiny cowbirds would, however, significantly alter our interpretation of the role of cowbird call structure in host manipulation. We therefore conducted the first quantitative test for such polymorphisms. For chicks of 4 and 8 days of age, we compared our samples of shiny cowbird calls reared by house wrens to the calls of same-age cowbirds recorded at nests of two other common hosts at our study site, the chalk-browed mockingbird (*Mimus saturninus*, 4 days: *n*=20 nests, 8 days: *n*=17 nests) and the rufous hornero (*Furnarius rufus,* 4 days: *n*=6 nests, 8 days: *n*=5 nests). Naturally-parasitized nests of mockingbirds and horneros were located by searching breeding territories. We manipulated mockingbird clutches prior to incubation to achieve broods of one cowbird and 2 mockingbird young (see Gloag et al. 2011 for details). In the case of horneros, we used unmanipulated broods containing one cowbird and between 0-3 hornero young. For three of the six hornero nests at which recordings were made, the nest already contained chicks at the time it was discovered and cowbird age was estimated based on body weight and tarsus length according to the growth curves in Gloag et al. (2011). The calls of cowbirds reared by mockingbirds and horneros were scored from spectrograms just as for those reared by wrens (described in Methods), to give a mean score from each recording of five variables: call duration (s), maximum and minimum frequency (Hz), peak frequency (Hz) and bandwidth (Hz). We then checked for host-specific differences between cowbird calls in two ways. Firstly, we performed multivariate comparisons of call structures using MANOVAs at each nestling age. Secondly, we performed discriminant function analyses (DFAs) at each nestling age. All variables for DFAs were entered, with the exception of frequency bandwidth which did not meet *a priori* criteria for minimum tolerance (>0.001, Costanza and Afifi 1979). Model fits were assessed via Wilk's lambda (Λ, and associated *p*), and the proportion of samples the model correctly assigned to host (i.e. the model's ability to discriminate between cowbird calls on the basis of host), using the cross-validation method. We found no evidence for differences in call structure between cowbird calls reared in different hosts at either 4 or 8 days of age, as shown in Table S1 below. Tests were performed in SPSS v. 20.0.

Table S1. Results of MANOVAs and DFAs comparing call structure of shiny cowbirds reared in three common hosts in Argentina: house wrens, chalk-browed mockingbirds and rufous horneros. MANOVAs used five call variables: call duration, maximum and minimum frequency, peak frequency and frequency bandwidth. DFAs used the first four of these five variables,.

	MANOVA			*DFA*			
n	*F*	*df*	*p*	Λ	*df*	*p*	*correctly classified*
DAY 4	1.2	8	0.31	0.75	10	0.38	26.2%
DAY 8	1.0	10	0.48	0.74	10	0.47	43.2%

(b) *Pairwise comparisons of shiny cowbird and house wren call parameters*

Table S2. Mean (± s.e.) values of acoustic structure parameters for the begging calls of cowbirds and house wrens at ages 4, 6, 8 and 10 days post-hatch, recorded *in situ* in naturally-parasitized nests, and the results of paired t-tests comparing the calls of each species. Significant differences, after adjusting α levels for multiple comparisons, are marked with an asterisk.

	House Wren	Shiny Cowbird	*t*	*d.f.*	*p*
DAY 4 (*n*=16)					
Call Duration (s)	0.05±0.003	0.13±0.016	5.01	15	<0.001*
Min. Frequency (Hz)	6721±165	4633±129	-8.95	15	<0.001*
Max. Frequency (Hz)	10475±274	7158±232	-8.39	15	<0.001*
Peak Frequency (Hz)	8413±156	6173±190	-9.34	15	<0.001*
Frequency Range (Hz)	3754±184	2524±145	-4.81	15	<0.001*
DAY 6 (*n*=14)					
Call Duration (s)	0.10±0.008	0.27±0.02	6.38	13	<0.001*
Min. Frequency (Hz)	6568±239	4660±124	-6.93	13	<0.001*
Max. Frequency (Hz)	11541±472	7131±272	-7.33	13	<0.001*
Peak Frequency (Hz)	8439±280	6155±216	-5.7	13	<0.001*
Frequency Range (Hz)	4972±340	2471±163	-6.27	13	<0.001*
DAY 8 (*n*=10)					
Call Duration (s)	0.11±0.4	0.33±0.2	8.4	9	<0.001*
Min. Frequency (Hz)	6334±410	4744±102	-3.9	9	0.003*
Max. Frequency (Hz)	10688±656	7803±221	-4.2	9	0.002*
Peak Frequency (Hz)	7774±374	6688±193	-2.5	9	0.033
Frequency Range (Hz)	4353±336	3058±136	-3.6	9	0.005*
DAY 10 (*n*=9)					
Call Duration (s)	0.13±0.06	0.42±0.02	19.3	8	<0.001*
Min. Frequency (Hz)	5702±445	4121±325	-4.05	8	0.004*
Max. Frequency (Hz)	10001±506	7789±347	-6.3	8	<0.001*
Peak Frequency (Hz)	7158±542	6508±306	-1.6	8	0.147
Frequency Range (Hz)	4299±220	3622±136	-2.3	8	0.045

Chapter 5

The economics of nestmate-killing in avian brood parasites: a provisions trade-off

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ABSTRACT

Some brood parasites kill all of their host's offspring shortly after hatching while others are 'tolerant' and are reared in mixed host-parasite broods. This difference may arise because nestling parasites face a 'provisions trade-off', whereby the presence of host nestlings can increase or decrease a parasite's food intake depending on whether host young cause parents to supply more extra food than they consume. We model this trade-off and show that the optimal nestmate number from a parasite's perspective depends on the interaction of two parameters describing a parasite's stimulative and competitive properties, relative to host young. Where these parameters differ from one host-parasite pair to the next, either nestmate-killing or nestmate-tolerance can be favoured by natural selection for maximum intake. We show that this extends to variation between hosts of generalist parasites. In an experimental field study, we found that nestling shiny cowbirds (*Molothrus bonariensis*) reared by house wrens (*Troglodytes aedon*) had higher food intake and mass growth rate when accompanied by host young than when alone, while those reared by chalk-browed mockingbirds (*Mimus saturninus*) had higher food intake, mass growth and survival when reared alone than with host young. In both hosts, total provisioning was higher when host nestlings were present, but only in house wrens did cowbirds secure a sufficient share of that extra provisioning to benefit from host nestlings' presence. Thus, a provisions trade-off might generate opposing selective forces on the evolution of nestmate-killing not only between parasite species but also within parasite species using multiple hosts.

1. INTRODUCTION

Obligate brood parasites lay eggs among other species' clutches and benefit from their hosts' misdirected parental effort. In many species, the young parasites attack and kill their host's offspring shortly after hatching; the fry of brood parasitic catfish eat host's eggs and fry whilst being brooded inside the mouth of the host Cichlidae (Sato 1986), cuckoo-wasp larva (Chrysidinae) devour the host larvae sharing their brood cell (Thomas 1962) and honeyguides (Indicatoridae) use bill hooks to mortally wound their nestmates (Ranger 1955). In the best-known example, chicks of brood-parasitic cuckoos (*Cuculus* sp.) dispose of host eggs or chicks from the nest by lifting them onto their backs and tossing them out of the nest cup (Jenner 1788). Such virulent behaviors in young parasites have long been considered adaptive as they eliminate competition for food and resources; as Darwin (1859) concluded with respect to cuckoo chicks' virulent behaviors, 'if it were of great importance for the young cuckoo, as is probably the case, to receive as much food as possible…those which had such habits and structure best developed would be the most securely reared.'

Among the avian obligate brood parasites however, roughly one third of all extant species have either not evolved brood-killing behaviors or have secondarily lost them (Davies 2000; Kilner 2005), an absence phenomenologically referred to as 'host-tolerance', or more accurately 'nestmate-tolerance', on the part of the parasite. In these cases, while adult parasites may reduce host clutches (via ruin or removal of eggs), some host offspring typically survive to be reared alongside the parasite (or parasites) in the nest. As the ancestral character, nestmate-tolerance might reflect the more recent origins of obligate parasitism in some lineages (i.e. evolutionary lag, Davies 2000) or be maintained by one or more constraints on the evolution of virulent behaviors in nestlings (e.g. energetic, Grim 2006; Grim et al. 2009a; Anderson et al.

2009, indirect fitness, Davies 2000, or host defenses, Broom et al. 2007; Grim et al. 2011).

A further, not mutually exclusive, explanation is that where selection favors the strategy that brings a parasite, in Darwin's words, 'the most food possible,' nestmate-tolerance will at times be maintained. In a study of brown-headed cowbirds (*Molothrus ater*) reared in nests of the Eastern phoebe (*Sayornis phoebe*), Kilner et al. (2004) found that host parents provisioned more to nests where cowbirds shared with two host chicks than to nests with lone cowbirds, that cowbirds in mixed broods took the lion's share of the provisions, and that they received more food and had greater growth than cowbirds reared alone in the nest. The explanation for this result may lie in nestling begging, which has a dual effect in most altricial bird species (Rodríguez-Gironés et al. 1996; Rodríguez-Gironés et al. 2001): first, it stimulates host parents to increase provisioning to the nest as a whole by intensifying foraging and/or reducing their own consumption (an effect of the begging output of the whole brood) and second, it determines the partitioning of the deliveries among the nestlings (an effect of begging of individual chicks). Thus to maximize their food intake and fitness, brood parasites might in fact face a trade-off between the advantage of removing host young, as competitors, and tolerating them, so as to gain the value of their assistance in stimulating host parents to work (Kilner 2003; Kilner et al. 2004). This trade-off (hereafter: 'provisions trade-off') could help explain the dichotomy between nestmate-killers and nestmate-tolerants among avian brood parasites, with differences in costs and benefits variously favoring the evolution of one strategy or the other (Kilner 2005).

In this study, we examine the provisions trade-off hypothesis both theoretically and experimentally. We first develop a model that formalizes the trade-off as the product of its component functions: the total provisioning rate stimulated by the whole brood and the share of those provisions received by a parasite nestling. The model proposes two qualities of a parasite nestling to be crucial in determining which scenario (host young present or absent) will optimize that parasite's intake. The first of these qualities is the relative responsiveness of host parents to own and parasite nestlings' begging. The second is the parasite's ability to compete during food allocation.

As both qualities are determined in relative terms on a host-parasite pair by pair basis, variation might exist not only between parasite species but within generalist parasites, those which use more than one host species at an individual or population level. Here the generalist cowbirds present a conspicuous example. Large size relative to host young afforded the nestling brown-headed cowbirds of Kilner et al.'s (2004) study a significant competitive advantage in the bidding war over their diminutive phoebe nestmates. Brown-headed cowbirds however use a wide-range of host taxa that vary in body size, incubation time and nestling growth rates (Friedmann and Kiff 1985; Lowther 1993; Remes 2006) and thus nestling cowbirds encounter a range of competitive brood environments (Kilner 2003; Remes 2010; Rivers et al. 2010). This raises the possibility that generalist cowbirds can encounter both scenarios in a provisions trade-off, optimizing food intake in some hosts when reared alone and in other hosts when host offspring are present. As such, these systems are of particular interest in assessing the role of a provisions trade-off in the evolution of nestling virulence. On one hand, they present a ready test of the central tenet of the trade-off, showing that a tolerant (i.e. ancestral-state) parasite could face differing selection pressures depending on the dynamics of its particular host-interaction. On the other hand, they raise questions about how selection for maximum food intake might be expected to act in generalists where the costs and benefits of nest-sharing vary between hosts.

We perform a field experiment with a generalist, nestmate-tolerant parasite, the shiny cowbird (*Molothrus bonariensis*). Like the North American brown-headed cowbird, this species is an extreme generalist, estimated to be reared by around 100 hosts representing a wide variety of taxa, body sizes, life histories and nesting ecologies (Lowther and Post 1999). We chose two representative hosts at extremes of the range of relative size: the house wren (*Troglodytes aedon,* hereafter: wren), a host smaller in body mass than cowbirds (11-14g and 40-45g respectively) and the chalk-browed mockingbird (*Mimus saturninus,* hereafter: mockingbird), a large-bodied host (70-75g). Following the design of Kilner et al. (2004), we compare, in each host, food intake, growth and survivorship of cowbirds reared in one of two treatment nests: a cowbird alongside host young and a cowbird alone. A previous study by Fiorini et al. (2009) found that shiny cowbirds reared in small host broods of mockingbirds had better survival and fledgling mass than those in larger broods, while larger host broods led to higher fledging masses for cowbirds reared by house wrens. Based on this previous study and our model, we hypothesize that nest-sharing with host young is advantageous to shiny cowbird food intake and growth when parasitizing house wrens but costly when parasitizing mockingbirds.

2. A MODEL

We model the intake of nestling parasites as a function of brood composition. The model includes the following assumptions:

(a) Total provisioning rate to a parasitized brood ($P_{T,}$ units of energy per unit time) is a function of total begging stimulation by the brood as perceived by the host parents (B_T, dimensionless). Begging stimulation here is used in its broadest sense to incorporate all stimuli provided by nestlings to elicit parental provisioning. Provisioning rate increases at a diminishing rate with increases in the brood begging stimulus, up to a maximum rate, P_{max}, the maximum food per unit time the parents are capable of delivering. The following exponential function, while not unique, has the required properties:

$$P_T = P_{max}(1 - e^{-mB_T}) \qquad (1)$$

where m is a dimensionless positive constant that scales the response of parents to changes in begging.

(b) Begging host and parasite chicks may not be equally effective stimuli for parental provisioning, such that total stimuli from the begging brood, B_T, results from the summed effects (without interaction) of host and parasite chick numbers weighted by their species-specific efficiency in stimulating host parents, B_h and B_p respectively:

$$B_T = N_h B_h + N_p B_p \qquad (2)$$

The relative value of B_h and B_p can be expressed as a parameter, β, the ratio between the strength of stimulation (provisioning increase per individual chick begging) produced by individual chicks of each species:

$$\beta = \frac{B_p}{B_h} \tag{3}$$

B_h and B_p, are abstract and dimensionless terms, that have the potential to incorporate various complexities of the begging stimulus, e.g. B_h could vary with the size and/or composition of the brood (Johnstone 2004; Pagnucco et al. 2008) or according to the sexes of the feeding adults (Macgregor and Cockburn, 2002; Tanner et al. 2008) or the presence of helpers (Wright 1998). For our purposes here however, we define a fixed B_h (=1) as the contribution to the overall begging stimulus made by one host chick. This condition allows us to express B_T in terms of the relative measure of a parasite's ability to stimulate provisions, β. Now, substituting equation (3) into equation (2):

$$B_T = N_h + N_p\beta \tag{4}$$

Then, substituting equation (4) into equation (1):

$$P_T = P_{max}\left(1 - e^{-m(N_h + N_p\beta)}\right) \tag{5}$$

(c) Once parents arrive with food at the nest, the food is distributed according to the competitive ability of each nestling. We define γ as the differential competitive ability of host (C_h) and parasite (C_p) nestlings, where competitive ability is the relative probability of consuming each food item at the time it is delivered:

$$\gamma = \frac{C_p}{C_h} \tag{6}$$

(d) Competitive ability may be influenced by nestling size, positioning in the nest, begging intensity and other variables that affect probability of feeding (Rodríguez-Gironés et al. 2001). When $\gamma=1$, all chicks in the nest receive an equal share of provisions. When $\gamma\neq1$, individual parasite nestlings receive greater or fewer feeds than individual host chicks. As each competitive unit will take the same fraction of the food brought up to the nest, we can use the competitive abilities of host and parasite nestlings to calculate the rate of food intake F (units of energy per unit time) for each parasite nestling as a proportion of the total provisions to the brood (P_T):

$$F = \left(\frac{C_p}{N_p C_p + N_h C_h}\right) P_T$$

$$= \frac{P_T}{N_p + \frac{C_h}{C_p} N_h} \tag{7}$$

Expressing the ratio C_h over C_p in terms of γ from equation (6) gives:

$$F = \frac{P_T}{\left(N_p + \frac{N_h}{\gamma}\right)} \tag{8}$$

And finally, substituting P_T from equation (5) into equation (8) gives:

$$F = \frac{P_{max}\left(1 - e^{-m(N_h + N_p\beta)}\right)}{N_p + \frac{N_h}{\gamma}} \tag{9}$$

The model is described graphically in Figure 1. In this plot, m is fixed at 0.5, representing a situation whereby provisioning rate gets close to its asymptotic value (P_{max}) with an unparasitized brood of five chicks (see Suppl. Material for a sensitivity analysis of m). The plots show that when a parasite is alone in the nest (N_p=1, N_h=0) host parents provision at a rate below their maximum capability ($P_T < P_{max}$) but the parasite receives 100% of provisions. With the addition of host nestmates, the total rate of provisioning increases following a negative exponential function, while the share received by the parasite decreases hyperbolically. Maximum intake can occur when host nestmates are absent (Figure 1a), or present (Figure 1b), depending on the parasite-host pair's relative parameters (β and γ). Thus, the model shows that both nestmate-killing and nestmate-tolerant strategies could maximise food intake of the parasite. The key lies in the size and fate of the marginal increase in provisioning generated by the presence of host nestlings: if each host nestling causes a greater increase in provisioning than the amount it consumes, then the presence of host chicks would result in higher consumption for the parasite, even if a host chick takes a bigger fraction of the extra food than the parasite.

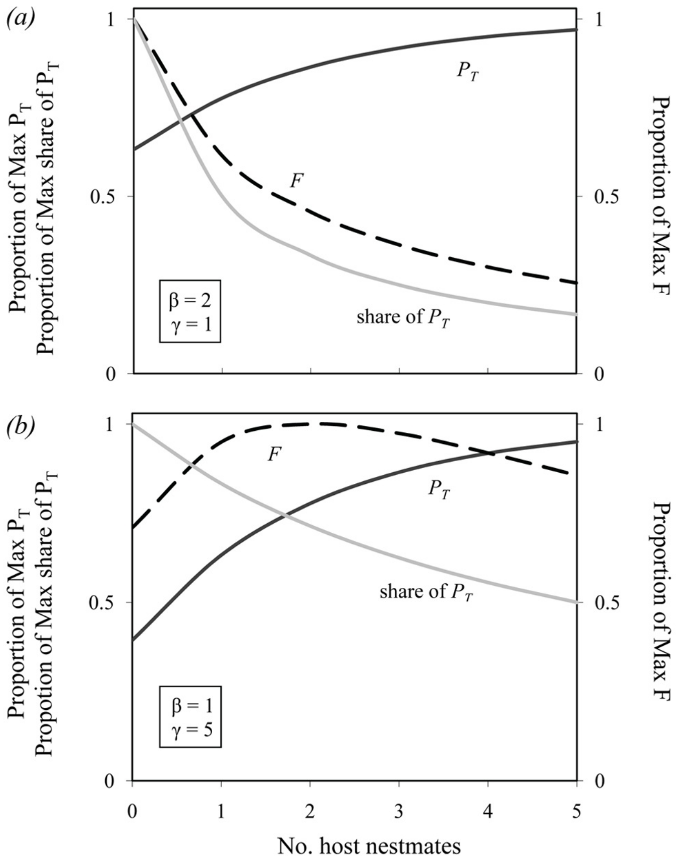

Figure 1. A model of the consequences for a brood parasite's food intake of sharing a nest with host young. Total provisioning (P_T), share of the total provisioning received by a nestling parasite (share of P_T) and thus food ingested by a nestling parasite (F) vary as a function of the number of host nestmates reared alongside the parasite. With increasing numbers of nestmates, P_T increases and share of P_T decreases (see main text for equations). Assuming that a single parasite chick is present, depending on the ability of the parasite to stimulate provisioning (β) and to compete for provisions (γ), F can be maximised either when: (*a*) parasites are alone in the nest, or (*b*) parasites share the nest with host young. β and γ values are inset, m=0.5.

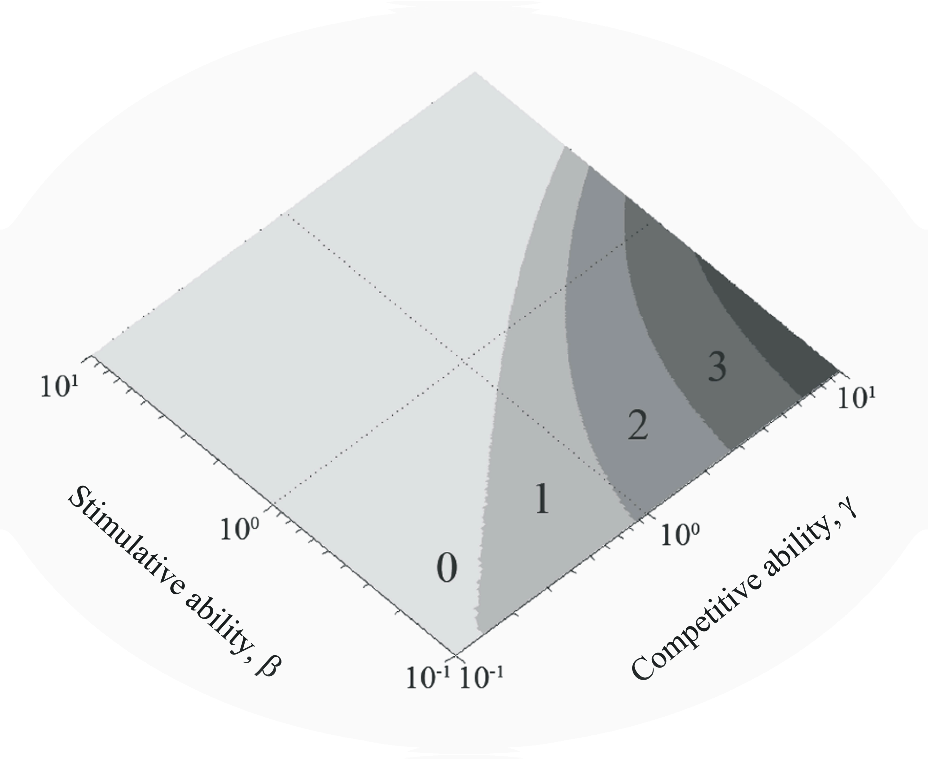

Figure 2. Optimal number of host nestmates, N_{opt} (colour-coded inset numbers 0-3), for a single parasite of given relative ability to stimulate host parents to provision (β) and to win those provisions when they arrive (γ) according to a model of a provisions trade-off (see main text). Axes are logarithmic, m=0.5. Dotted lines indicate β=1 and γ=1, thus their intersection is the point at which parasite and host nestlings are equally matched in both begging dimensions.

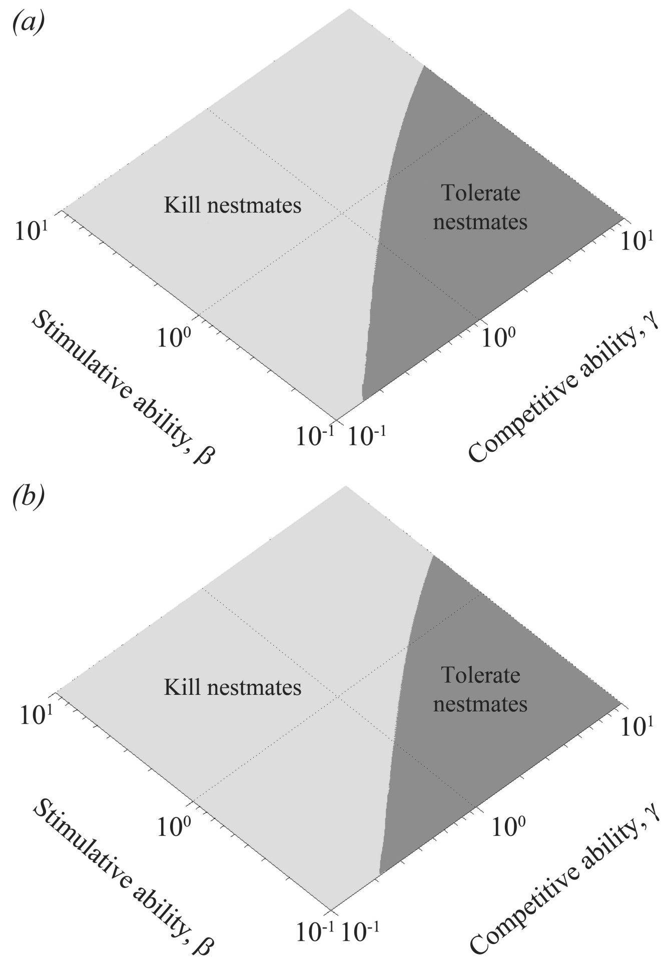

Figure 3. The adaptiveness of nestmate killing over nestmate tolerance for a single parasite chick according to a model of a provisions trade-off (see main text). The plots assume a given host brood size of either (*a*) 2 host chicks, or (*b*) 4 host chicks. Dotted lines indicate β=1 and γ= 1. The range of parameters for which nestmate-tolerance maximises food intake is larger when the number of host young in the brood is lower, though for both brood sizes, if β is low and γ high, maximum intake occurs when the single parasite chick does not kill its nestmates (dark grey), and if β is high and γ low, selection favors nestmate-killing (light grey). The figure shows that typical host brood size, in addition to begging and competitive abilities, would influence the evolution of nestmate-killing behavior.

Restricting ourselves for the moment to a situation in which there is only one parasite in a nest (N_p=1), we can use equation (9) to find the optimal number of host nestmates (N_{opt}) for the parasite, defined as the number of host nestmates ($0 \leq N_h \leq 10$) for which the model predicts that the parasite will enjoy maximum intake (calculations performed in MATLAB, Mathworks, Release 2010a). Figure 2 shows N_{opt} across the range of values (0-1) of β and γ (and m=0.5). From these calculations, we can see clearly that neither stimulative nor competitive ability alone determine the outcome of the trade-off, but rather that their interaction is paramount. When $\beta > \gamma$ a parasite's relative stimulative ability is greater than its relative ability to win intrabrood competition for provisions, and parasites maximize food intake when reared alone. This includes the situation where parasites are equivalent to host young in both respects, $\beta=\gamma=1$, parameter values that also indicate the outcome for intraspecific parasites, or for interspecific parasites whose only nestmates are conspecific parasites (as can occur when multiple parasitism is common). In contrast, for many (though not all) cases when $\gamma > \beta$, parasites will benefit from the survival of some host offspring and receive maximum food when sharing the nest.

Optimal nestmate number however, which assumes any number of nestmates is possible, may be an unlikely target for natural selection. We can in addition use the model to ask under what conditions a parasite nestling would benefit from either killing or tolerating its nestmates, where parasites are bound by a binary rule of kill all (and be reared alone) or kill none (and be reared alongside all host young that survive to hatch). Here, typical host brood sizes and the extent of host brood reduction enacted by adult parasites must play a role. Figure 3 shows these calculations for nestling parasites facing two illustrative host brood sizes, either two or four. Nestmate-killing is favored over a larger range of parameters when there are more host young in the brood. For both brood sizes however, as demonstrated in the calculation of optimal nestmate number, we find that both the presence or absence of host nestmates can result in maximum food intake. Thus we predict that where parasite-host pairs vary between each other in relative competitive and/or relative stimulative ability they could be subjected to opposing selective forces on the evolution of virulent behaviors at the nestling stage. We now describe a field experiment designed to identify such circumstances, by examining the performance of nestlings of a generalist parasite, the shiny cowbird, in nests of two common hosts between which the relative competitiveness of the parasites is expected to vary markedly.

3. METHODS

(a) *Study species and field procedures*

Chalk-browed mockingbirds and house wrens are both common hosts of shiny cowbirds in southern South America. We searched for mockingbird nests in trees and shrubs of known breeding territories, and checked nestboxes erected for wren use at our field site, Reserva El Destino, an estate of approximately 600ha (35°08′S, 57°23′W) located within the Biosphere Reserve (UNESCO) Parque Costero del Sur, Buenos Aires Province, Argentina. Fieldwork took place during the breeding seasons 2008-2010. Nests were located prior to the onset of incubation. In each of the two host species we created two treatment groups; ‘mixed’ (a cowbird reared alongside host young) and ‘alone’ (a cowbird reared alone).

Mockingbirds lay clutches of 4-5 eggs but egg-puncturing by adult cowbirds at or around the time of parasitism reduces clutch size for parasitized nests. At our field site the mean number of mockingbird young in parasitized nests at hatching

(±s.e.) is 1.7±0.2 (Fiorini 2007). Multiple parasitism is common in this host, thus cowbirds can share the nest with other cowbirds, but our experiment focused only on the effects of host nestmates. Thus in mockingbirds, nests were manipulated to contain either two mockingbird eggs and one cowbird egg ('mixed') or two 'dummy' plaster-of-Paris eggs and one cowbird egg ('alone'); *n*=20 per treatment (2008/9: *n*=5, 2009/10: *n*=15 per treatment). On six occasions, when eggs failed to hatch or were destroyed during incubation by cowbirds, we moved chicks between mockingbird nests shortly after hatching to create mixed brood treatment nests.

In wrens, the number of host young reared alongside cowbirds in natural conditions depends not only on loss from egg-puncturing (initial clutch size of 4-5) but on the synchrony of parasitism with host laying; synchronous parasitism leads to cowbirds hatching earlier than host young and quickly outcompeting them to death, while asynchronous parasitism (when cowbirds lay after the onset of incubation) can lead to a same-age brood in which both parasite and hosts fledge (Tuero et al. 2007). At our field site, asynchronous parasitism and host nestling survival is not uncommon (Fiorini et al. 2009). In order to investigate the effect of nest-sharing on the food intake of cowbirds reared in wrens our 'mixed' treatments were therefore created by simulating asynchronous laying by adding cowbird eggs to clutches of three or four host eggs 3-days after the onset of incubation such that host and parasite hatched at the same time. Nests in the 'alone' treatment were handled similarly but either had all host eggs replaced by dummy eggs before incubation, or (in four cases) host young translocated to other nests within 24 hours of hatching: *n*=15 per treatment (2008/9: *n*=3, 2009/10: *n*=5, 2010/11: *n*=7 per treatment).

Shiny cowbirds are sexually size dimorphic (Friedmann and Kiff 1985). Post-hoc molecular sexing showed that male and female cowbirds had been assigned approximately equally between treatments by chance (but see Suppl. Material for an analysis of possible sex effects).

(b) *Analysis*

We filmed each nest for 2-3 hours when the cowbird chick was 4 and 8 days post-hatching (day of hatching = day 0) using a microcamera suspended above the nest and connected to a Digital Personal Video Recorder (Handykam: JXD990) at the base of the tree. Some nests were not recorded on one or both days due to chick death, adverse weather or, in wrens, because nests were built too close to the nestbox ceiling. All host parents resumed feeding within 30 mins of placing the camera. We analyzed the final hour of each recording for: i) number feeding visits (i.e. visits where the host parent brings food), ii) number of feeding visits in which the cowbird received food and, iii) the size of the prey (% bill width of host parent, abbreviated %BW). We used analysis of variance and non-parametric equivalents (Mann-Whitney U test) to test for differences in these variables between treatments.

We measured chick body mass (g) and tarsus length (minimum tarsus, mm, Redfern and Clark 2001) from days 0-10 (cowbird fledging occurs day 11-12 in mockingbirds (Fiorini et al. 2009) and day 12-14 in wrens (Kattan 1996)). We used Chi-square tests to test for differences in chick mortality rate between treatments. Cowbird chick mortality was recorded as independent of predation when either i) chicks were found dead in the nest (*n*=7) or ii) they disappeared from the nest after failing to increase in weight in the two days prior to disappearance (*n*=3). In the latter case it was likely they had died in the nest and been removed by host parents. Only chicks that survived at least 8 days were included in the growth rate analysis. To test for differences in growth rates of body mass and tarsus length between treatments (with or without hosts) we used nonlinear regression to fit growth curves of each chick to the logistic equation:

$$M_t = \frac{A}{1 + e^{-K(t-t_i)}}$$

where M is the mass (g) or tarsus length (mm) of the chick at time t, A is the asymptotic mass or tarsus length, t is the time since hatching (hours, where hatching hour was designated as sunrise of day 0), t_i is the inflection point of the curve (hours) and K is the growth constant (hours^{-1}) i.e. the rate constant of the logistic curve (Ricklefs 1967). A was fixed at the maximum mass or tarsus length measured to allow comparison of K values between treatments (Starck and Ricklefs 2003). We then used general linear modeling for each of four dependent variables; growth rate of body mass and growth constant of tarsus (K for both variables), mass at 8 days post-hatch and tarsus length at 8 days post-hatch. GLMs tested the effect of treatment ('mixed' vs. 'alone') on each growth variable for each host (GLM Univariate; Type III sums of squares).

In addition, we tested for differences between hosts but within treatments, to assess relative impacts of nest-sharing on cowbirds in each host (including year as a factor; GLM Univariate). All statistics were performed in SPSS 17.0. All errors are given as ± one standard error of the mean.

4. RESULTS

(a) *Shiny cowbirds reared by house wrens*

Wrens provisioned more to broods comprising of a cowbird and their own young than to a lone cowbird at both 4 and 8 days (day 4: $F_{1,17}$=17.45, p=0.001, day 8: $F_{1,14}$=28.6, p=0.009; Figure 4). At both ages, cowbirds reared in mixed broods were larger than their nestmates (day 4: cowbirds 21±1.3g, wrens 6.5±0.3g, t_{14}=-11.9, p<0.001, day 8: cowbirds 36.8±1.3g, wrens: 11±0.3g, t_{11}=-18.5, p <0.001) and received a greater proportion of food deliveries than expected by chance (day 4: χ^2=63.5, p<0.001, day 8: χ^2=59.4, p<0.001; Figure 4). At 4-days, food intake for 'mixed' cowbirds was not significantly different from that of lone cowbirds reared in wren nests ($F_{1,17}$=0.19, p=0.61), but by 8 days, cowbirds sharing the nest with wren chicks received significantly more food than their counterparts reared alone ($F_{1,14}$=5.85, p=0.03). Prey size did not differ significantly between treatments at either age (day 4: mixed 282±5%BW, alone 244±32%BW, $F_{1,14}$=2.46, p=0.63, day 8: mixed 363±15%BW, alone 348±27%BW, $F_{1,8}$=2.75, p=0.44).

Differences in food intake were reflected in differences in growth. Cowbirds reared alongside wren nestlings had higher rates of mass and tarsus growth (mass growth rate: mixed K=0.021±0.001 hours^{-1}, alone K=0.016±0.001 hours^{-1}, $F_{1,24}$=4.87, p=0.037; tarsus growth rate: mixed K=0.018±0.001 hours^{-1}, alone K=0.016±0.001 hours^{-1}, $F_{1,24}$=4.7 p=0.04); and reached higher day 8 body mass than those reared alone in wren nests ($F_{1,24}$=5.0, p=0.035), though we found no significant difference in tarsus length at day 8 ($F_{1,24}$=2.9, p=0.13; Figure 5). The non-predation mortality of cowbirds reared in wren nests did not differ significantly between treatments (mixed: 2 of 16, alone: n=2 of 13, χ^2=0.031, p=0.86).

(b) *Shiny cowbirds reared by mockingbirds*

Mockingbirds also provisioned more to mixed broods than lone cowbird broods at 4 and 8 days post-hatch (day 4: $F_{1,32}$ =11.97, p=0.002, day 8: $F_{1,17}$ =22.15, p <0.001; Figure 4). At 4 days, cowbirds reared alongside mockingbird young were not significantly different in mass (cowbirds 19±1.7g, mockingbirds 22±1.8g, t_{19}=1.54, p=0.14) nor competitiveness from their host's young (i.e. proportion of feeds secured, χ^2=0.01, p=0.91) but received significantly less food deliveries per hour than their counterparts reared alone ($F_{1,32}$=4.56, p=0.04; Figure 4). By 8 days, cowbirds cohabiting with mockingbirds weighed significantly less than their nestmates (cowbirds: 30.2±2g, mockingbirds: 42.7±2g, t_8=3.84, p=0.001) and received food on fewer feeding visits than expected by chance (χ^2=10.8, p<0.001; Figure 4). Eight-day old cowbirds alone in mockingbird nests received almost twice as many feeds per hour as those cohabiting with host young ($F_{1,17}$=4.55, p=0.048; Figure 4). Prey size did not differ significantly between treatments at either age (day 4: mixed 303±21%BW, alone 302±14%BW, Z=-0.87, p=0.38, day 8: mixed 387±22%BW, alone 413±33%BW, Z=-5.7, p=0.57).

In mockingbird nests, cowbird nestling mortality was lower for cowbirds reared alone than for those reared alongside host chicks (excluding predation, mixed: n=6 of 14, alone: n=0 of 14, χ^2=7.63, p=0.006). Furthermore, cowbirds reared alone in mockingbird nests had higher body mass growth rates and reached a higher mass at 8 days than those that survived sharing the nest with mockingbird young (mass growth rate: mixed K=0.014±0.001 hours^{-1}, alone K=0.018±0.001 hours^{-1}, $F_{1,23}$=5.19, p=0.032, mass 8-days: $F_{1,23}$=5.48, p=0.028), though we found no significant difference between these treatments in growth rate of tarsus, or length of tarsus (mm) at 8 days (tarsus growth rate: mixed *K*=0.014±0.001 hours^{-1}, alone *K*=0.016±0.001 hours^{-1}, $F_{1,23}$=0.002, *p*=0.96, tarsus 8-days: $F_{1,23}$=0.22, *p*=0.64; Figure 6).

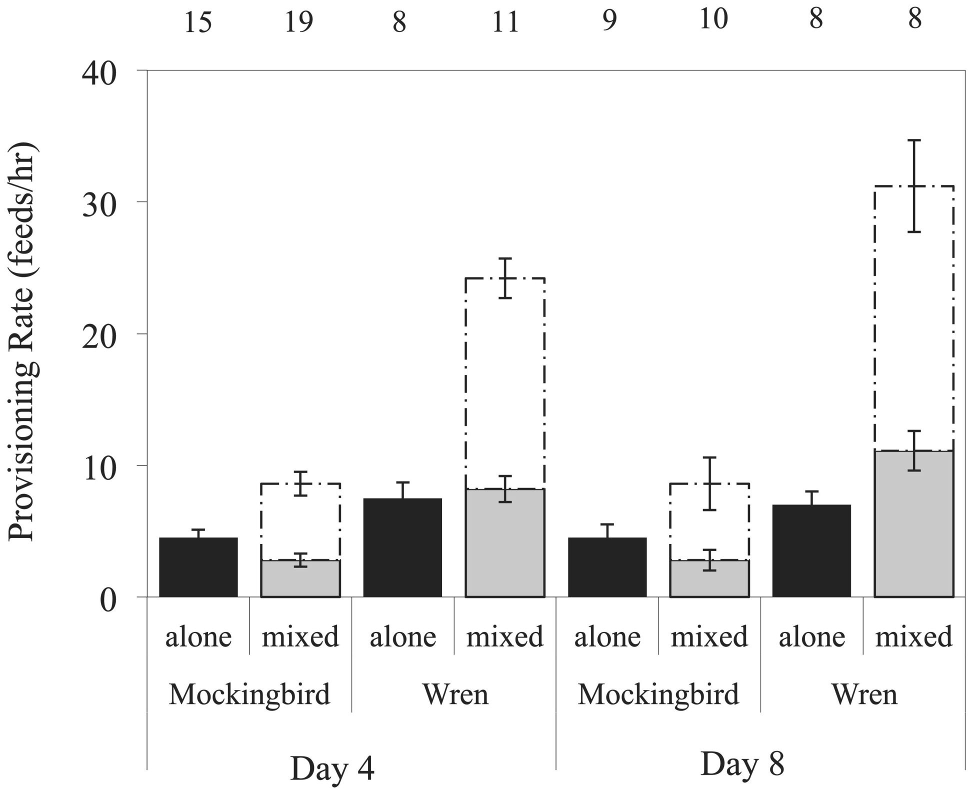

Figure 4. Provisioning rates (feeds/hr) to shiny cowbird nestlings reared alone (black bars) and reared alongside host chicks (grey bars) in either chalk-browed mockingbirds or house wrens at 4 days and 8 days post-hatch. Dashed bars indicate the total provisioning to the mixed broods, where host chicks received the remainder. Values are means ± s.e. Sample sizes, *n*, given above bars. See main text for statistical details.

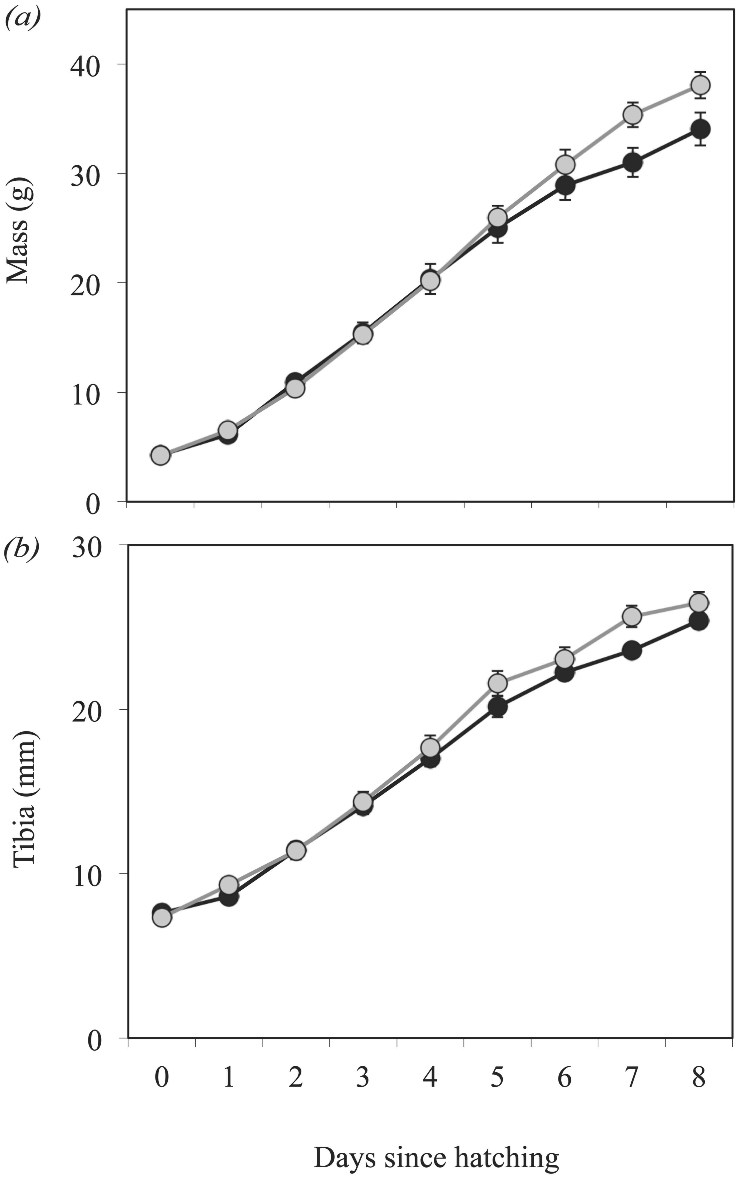

Figure 5. Growth in (a) body mass and (b) tarsus length of shiny cowbird nestlings reared alone (black circles, *n*=12) and reared alongside two host chicks (grey circles, *n*=14) in nests of house wrens. Values are means ± s.e.

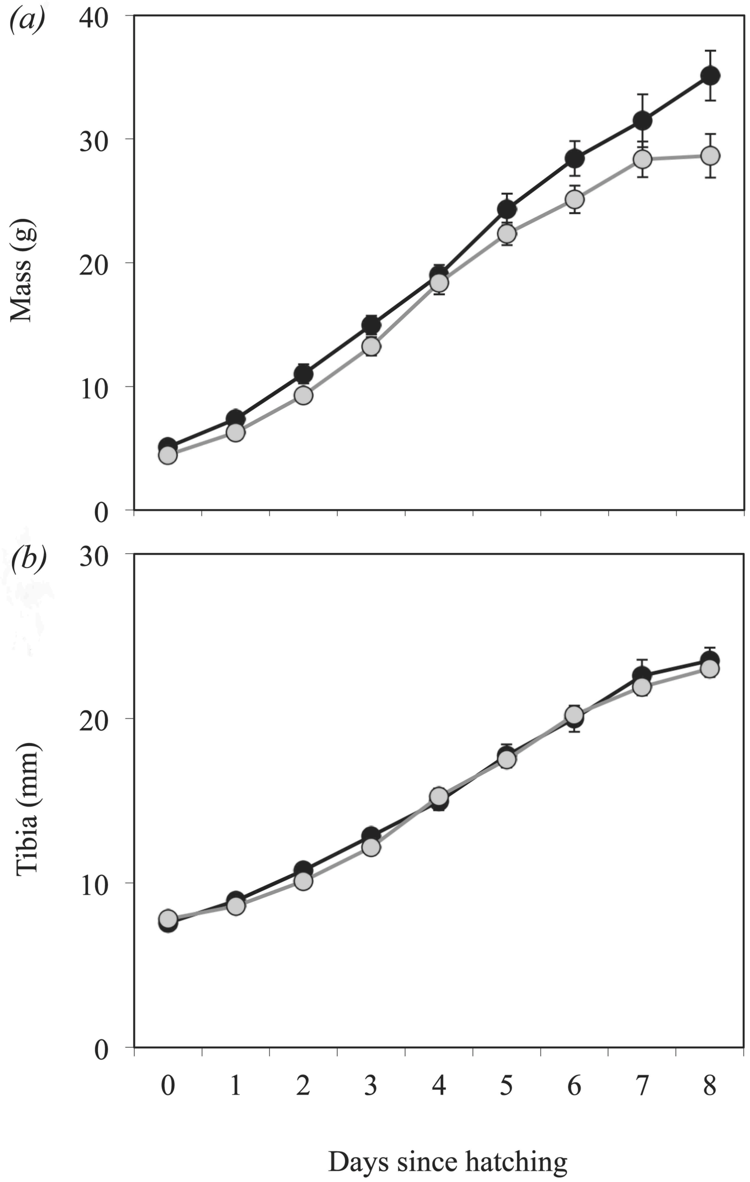

Figure 6. Growth in body (a) mass and (b) tarsus length of shiny cowbird nestlings reared alone (black circles, *n*=14) and reared alongside two host chicks (grey circles, *n*=11) in nests of chalk-browed mockingbirds. Values are means ± s.e.

(c) ***Between hosts comparisons***

Cowbirds reared in mixed broods in wrens had better mass and tarsus growth than those reared in mixed broods in mockingbirds (GLM, mass growth rate: $F_{4,20}$=4.2, p=0.012, tarsus growth rate: $F_{4,20}$=7.98, p=0.001) and reached greater weights and tarsus lengths by day-8 (mass: $F_{4,20}$=9.19, $p<0.001$, tarsus: $F_{4,20}$=10.02, $p<0.001$; Figures 5 and 6). There was no significant difference between cowbirds of the lone treatment in each host (GLM, mass growth rate: $F_{4,21}$=0.42, p=0.79, tarsus growth rate: $F_{4,21}$=0.45, p=0.77, mass: $F_{4,21}$=0.21, p=0.92, tarsus: $F_{4,21}$=0.86, p=0.5; Figures 5 and 6). There was no significant difference in cowbird chick survivorship between host species in either treatment (mixed: χ^2=2.8, p=0.09, alone: χ^2=2.53, p=0.11).

5. DISCUSSION

A provisions trade-off

Following reports that nestlings of some avian parasites experience a net growth benefit from the survival of their host's nestlings, interpreted as one side of a trade-off for maximum provisions (Kilner et al. 2004; Kilner 2005), we developed a model of the consequences to food intake that parasite nestlings might experience by sharing the nest with host offspring. The model considers that chick begging has two effects, one shared with the rest of the brood (increase in parental provisioning rate) and one exclusive to the begging chick (better chance of capturing food when delivered). A parasite's intake is therefore expressed as the product of total brood provisioning and the parasite's share of those provisions. Assuming selection acts to favor higher intake, our model thus helps to frame the potential routes by which nestmate-killing can evolve in avian parasites.

At the crux of the model are two assumptions: that total provisioning is likely to be a concave function of brood size asymptoting at the maximum provisioning potential of the host parents, and that food sharing is likely to be a ratio function with brood size included in the denominator. We further assumed that total provisioning depends on the number and stimulating power of the begging displays by both parasite and host nestlings and that the distribution function will depend on the competitive ability of such nestlings. All assumptions draw from empirical studies in the wider literature on avian begging (e.g. Wright and Leonard 2002), but they are of course generalizations and the extent to which they are appropriate for any one system will vary.

Our resulting model shows that either host killing or host tolerance can lead to higher parasite intake, depending principally on the interaction of the parasite's competitive advantage and the effectiveness of its begging signal in stimulating host parents to work. If parasites are equivalent to host young in both competitiveness and stimulating efficiency they receive most food when they are alone in the nest. This is also frequently true for host chicks in the absence of parasitism, because parental increase in provisioning is sub-linear with respect to brood size, but in the case of parasitism there is typically no kin-mediated benefit to offset the loss of intake caused by extra nestlings. When parasites are very effective at stimulating provisioning and/or relatively ineffective in contesting the food delivered, selection for maximum intake should similarly favor nestmate-killing. The opposite however (nestmate-tolerance) is expected whenever parasites have lower stimulating efficiency than host young and/or are good intrabrood competitors.

Variation in these qualities between ancestral parasitic lineages could arise simply from differences in the life-histories of the species involved. Based on empirical data from both parasitic and non-parasitic systems, body size (Lichtenstein 1998, Rivers 2007; Rivers et al. 2010), egg size (Forbes and Wiebe 2010), incubation times (Ostreiher 1997; Hauber 2003), growth rates (Werschkul and Jackson 1979; Kilpatrick 2002; Remes 2010) and brood sizes (Leonard et al 2000; Neuenschwander et al 2003; Fiorini et al 2009) are all likely to affect the relative competitive and signaling abilities of any one host-parasite pair. As hosts and parasites co-evolve, derived traits of either party could help drive selection in one or the other direction. As such, and as is the case with any evolutionary modeling, contrasting the model's predictions with observed behavior in present-day species is not straightforward. For instance, parasites may secondarily lose or gain adaptations for begging in response to the presence/absence of host nestmates resulting from their behaviors. Also, host nestlings may evolve strategies to better compete with parasites, such as exaggerated begging (an interaction effect not included in our model, but see Pagnucco et al. 2008; Boncoraglio et al. 2009). Bearing this in mind, we nevertheless see some evidence that differences in nestling virulence of extant parasites match the model's predicted trends in chick competitive and stimulative abilities. Nestmate-killing common cuckoos possess begging calls capable of eliciting provisioning rates from their reed warbler hosts equivalent to a whole brood of host young (Kilner et al. 1999). Similarly, nestmate-killing Horsfield's hawk-cuckoos display a wing-patch to host parents to superstimulate provisioning rates (Tanaka and Ueda 2005). Also consistently with the model, common cuckoos have been shown to compete poorly when forced to share the nest with host nestmates in experimental manipulations (Martin-Galvez et al. 2005; Hauber and Moskat 2008; Grim et al. 2009b). On the other hand, several nestmate-tolerant parasites compete well for food against their host nestmates; *Vidua* sp. finches are larger and hatch earlier than their host's offspring which likely gives them a competitive advantage during food allocation (Davies 2000), and the glandular markings inside the gapes of great spotted cuckoos (*Clamator glandarius*) increase their competitive ability by triggering preferential feeding from their magpie host parents (Soler et al. 1995).

Nestmate-killing and the generalist's dilemma

In the case of generalist parasites, the use of multiple hosts adds interesting complications because it is obvious from the model that the relative advantages of (and hence selection for) nestmate-killing and nestmate-tolerance must by necessity differ between different hosts of the same parasite. In our field study, nestmate-tolerant shiny cowbirds encountered both sides of a provisions trade-off, depending on the host used. When reared by chalk-browed mockingbirds, nestling cowbirds had higher food consumption, mass gain and survival when alone in the nest than when sharing with two mockingbird young. In contrast, cowbirds reared in the nests of house wrens had higher food intake and growth when reared alongside three or four host young than when reared alone.

Factors other than food intake may have contributed to differences in growth rates. For instance, nestlings of unparasitized broods help each other to thermoregulate, thus sometimes benefiting from larger brood sizes (e.g. Dunn 1976). However the differences in provisioning rates we observed from video data indicate food intake was likely the most significant driver of differential growth.

Our opposing results between the two host species are consistent with the predictions from a trade-off with respect to a parasite's competitive ability. In both hosts, cowbirds in mixed brood treatments elicited higher total provisioning rates than did lone cowbirds but only in house wren nests were cowbirds able to secure a sufficiently large share of these provisions to benefit from nest-sharing. Nestling size is probably key in these interactions, since shiny cowbirds were considerably larger than their wren nestmates but similar in size or smaller than their nestmate mockingbirds. Other studies on cowbird begging also suggest nestling size to be important in intrabrood competition, either because host parents preferentially feed larger nestlings or because large nestlings can best jostle their nestmates out of the way when food is on offer (Lichtenstein and Sealy 1998; Dearborn et al. 1998; Lichtenstein 2001; Kilner et al. 2004; Rivers 2007; Rivers et al. 2010). Competitive ability however, as our model shows, is only one side of the coin; it may be that differential stimulative ability of shiny cowbird nestlings between hosts also contributed to the observed outcome in this study, if say, cowbirds are better at soliciting mockingbird parents to provision (in our model, higher β) than they are at soliciting wrens.

How then might selection for maximum provisions act in shiny cowbirds and other generalist parasites in which the optimal host nestmate number varies from host to host? One possibility is that such parasites may evolve (or retain) the behavior that achieves an overall growth advantage given the relative incidence of host species used. Nestmate-tolerance may be better than nestmate-killing in some hosts and suboptimal in others but be maintained by the average effect, weighted by relevant properties of each host species such as maximum provisioning rate, food quality and length of the nestling period (Kilpatrick 2002; Remes 2010), as well as by the prevalence of certain host defenses (Langmore et al. 2003; Broom et al. 2007; Grim et al. 2011). The frequency of multiple parasitism may also be crucial, both because it may lead to parasites sharing with siblings or half-siblings, and because it changes the begging profile and competitive interactions of the brood. Multiple parasitism is common in a number of hosts of nestmate-tolerant parasites (Arias de Reyna 1998; Ortega 1998), including chalk-browed mockingbirds (Fraga 1985).

A 'general optimum' solution would account for the fact that the optimum in any one host is not necessarily equal to the optimum of another. We might predict for example that large hosts would deliver more or larger prey than smaller hosts (e.g. Grim 2006) resulting in a situation whereby a parasite had similar growth and survival in the suboptimal nestmate configuration of a large host than in the optimal one for a small host (Kirkpatrick 2002). Indeed, shiny cowbirds enjoy similar fledging success in large hosts as they do in small hosts (Sackmann and Reboreda 2003; Fiorini et al. 2005), which would be consistent with this effect. In our experiment, a comparison of cowbird growth between species in fact shows the opposite trend, with both groups of cowbirds reared in wrens (mixed and alone) achieving growth rates as good or better than those observed in the highest-growth (i.e. lone cowbird) treatment in mockingbirds. However, in practice the benefits of host-tolerance to wren-reared cowbirds will be considerably less because naturally-occurring parasitism is often synchronous with wren laying (Fiorini et al. 2009), and results in the 'passive' death of host young by starvation or trampling early in the nestling period (Tuero et al. 2007). Thus, within the particular pair of hosts we investigated, the host in which cowbirds most benefit from nestmates is also the host in which nestmates are least likely to be present.

A second possible solution to the generalist's dilemma of optimal nestmate number would be for parasites to adjust their virulent behaviors according to their host, either facultatively at the individual level or via the evolution of host races targeting certain hosts or host types (Gibbs et al. 2000; Mahler et al. 2007) and having host-appropriate virulent behaviors (Kilner 2005; Fiorini et al. 2009). Differential nestling virulence, where parasites kill nestmates in some hosts and not in others, is as yet unknown among today's parasites but could in theory arise if nestling aggression were triggered by host-specific stimuli or maternally inherited from host-specific females. Some evidence for host-adjusted virulent behaviors does however come from studies of clutch reduction by adult parasites. In cowbirds, adult females either remove or puncture host eggs at or around the time of parasitism (Ortega 1998). Fiorini et al. (2009) showed that shiny cowbirds puncture more eggs when parasitizing mockingbirds than house wrens even though the latter are easier to break. Brown-headed cowbirds have similarly been proposed to remove eggs more frequently in large hosts than small hosts (Davies 2000). In cases where nestlings' competitive ability is predictable from host-parasite relative egg size, as it likely is in cowbirds (e.g. Rivers 2007), this differential clutch reduction by the adult parasites could arise from the parasite female's simple rule of destroying more eggs when they are larger than her own and fewer when they are smaller. Adjustable clutch reduction by adult parasites may be a way to achieve optimal brood compositions for nestling parasites in cases where partially-reduced brood sizes lead to maximum intake. Our model indicates that intermediate brood sizes can indeed be optimal under some parameter values (see Figure 2). Importantly however, while adult virulent behaviors implies greater flexibility than is possible for a nestling bound by a kill-all nor kill-none rule, clutch reduction at the egg-stage is very likely to have upper limits, beyond which hosts abandon their nests (Winkler 1991; Anderson et al. 2009). Also, opposing selective pressures on clutch reduction may be at play, such as optimizing incubation efficiency (McMaster & Sealy 1997). Indeed, in any host-parasite system, selection for better provisioning is unlikely to be the only factor driving the evolution of virulent behaviours; a range of other candidate costs and benefits exist (e.g. Grim

2006). Future empirical studies of nestling intake in a variety of host-parasite pairs will reveal whether, amongst all these potential factors, a provisions trade-off has played a primary role in the evolution of nestmate-killing by parasites specifically, and host brood-reduction by parasites generally.

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8. SUPPLEMENTARY MATERIAL

(a) *Sensitivity analysis for the effect of m on optimal nestmate number*

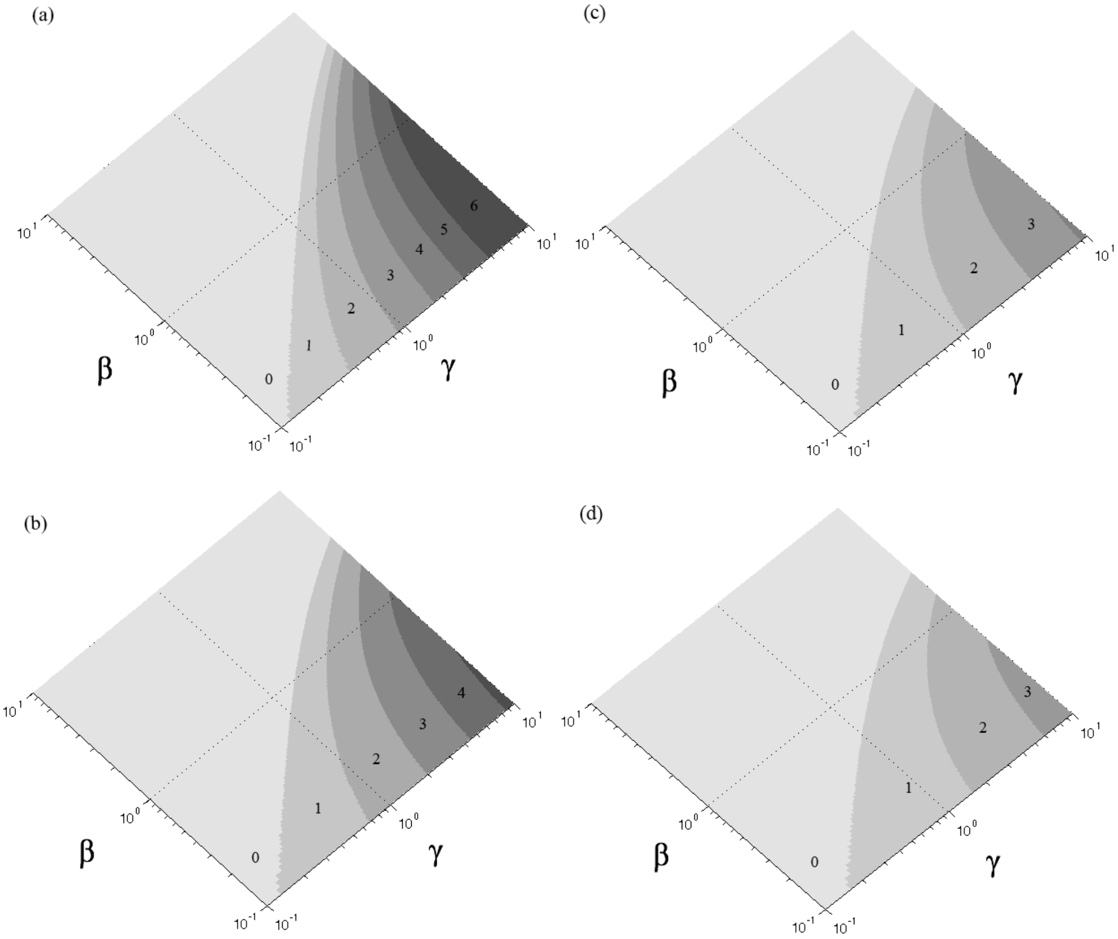

Figure S1. The host nestmate number (inset numbers) that maximizes intake of a single brood parasite nestling: (a) m = 0.8, (b) m = 0.6, (c) m = 0.4, (d) m = 0.2. The parameter m scales the response of host parents to increases in the brood begging signal. This sensitivity analysis shows that the qualitative predictions of the model are robust with respect to the parameter m.

(b) ***A test for an effect of cowbird chick sex***

Shiny cowbirds adults are sexually size dimorphic. At our field site, adult females and males weigh approximately 45g and 50g respectively (Fiorini et al. 2009), and both sexes fledge before they reach adult mass (Kattan 1996). To assess whether chance bias in the sex of eggs allocated between treatments could have influenced our comparison of growth rates we collected blood from cowbird chicks at 6 days of age and determined chick sex using standard molecular techniques (Griffiths et al. 1998). In some cases poor sample quality prevented successful sexing. Sexing data for cowbirds reared in mockingbirds was as follows: ‘mixed’ treatment: male=8, female=7, undetermined=5, ‘alone’ treatment: male=6, female=8, undetermined=6. Sexing data for cowbirds reared in house wrens was as follows: ‘mixed’ treatment: male=6, female =8, undetermined=3, ‘alone’ treatment: male=5, female=5, undetermined=5. In our samples of known sex, we found no significant difference between the sexes in growth rates (K) of mass (in mockingbirds: Mann Whitney U test: $Z = -1.426$, $p = 0.154$, in wrens: independent samples: $t_{21}= 1.3$, $p = 0.21$) or tibia (in mockingbirds: $t_{15} = 1.683$, $p = 0.113$, in wrens: $t_{21}= 1.35$, $p = 0.22$).

Chapter 6

Shiny cowbirds share nests, but not mothers, in multiply parasitized mockingbird broods

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ABSTRACT

The young of some brood parasitic birds do not kill the host's offspring sharing their nest, despite the potential gains of eliminating all competitors for parental care. Indirect fitness costs might account for such 'nestmate-tolerance' if females routinely laid more than one egg in the same host nest, as young parasites would otherwise risk killing their siblings along with any host chicks. We investigate this possibility in the shiny cowbird (*Molothrus bonariensis*) parasitizing a common host, the chalk-browed mockingbird (*Mimus saturninus*). In our study population, 89% of all cowbird eggs occur in multiply parasitized nests, but it is unknown whether one or several females are responsible for eggs in the same nest. We used remote video cameras to film egg-laying by individually marked females. Shiny cowbird eggs are highly polymorphic and recordings confirmed that each female lays eggs of a consistent appearance. Using both direct video evidence and indirect evidence from egg morphs, we established that different females were responsible for 91% or more of the eggs laid into already-parasitized nests, with up to 8 different females parasitizing the same nest. Thus while shiny cowbird chicks will frequently share a mockingbird's nest with conspecifics, these are rarely, if ever, their maternal siblings. We suggest that in the absence of indirect fitness costs, multiple parasitism might nevertheless have consequences for the evolution of virulent behaviours in nestling parasites, as it defines the composition of the brood in which parasites must compete.

1. INTRODUCTION

Obligate brood parasitic birds lay their eggs in the nests of other species, which then rear the young intruder at their own cost. Nestling parasites possess a suite of adaptations that ensure they secure from their adopted parents the best possible care. In some parasites, this includes eliminating the host's own offspring entirely, either by throwing them out of the nest (as do many Old World cuckoos, *Cuculinae*) or fatally stabbing them with hooked beaks (honeyguides, *Indicatoridae,* and American ground cuckoos, *Neomorphinae*). Thereby, the young parasite removes all competition for space and resources, becoming the sole occupant of the nest and sole recipient of all food delivered.

Not all parasitic species however display such ruthless behaviours as nestlings. Some can be described, in contrast, as 'nestmate-tolerant', and are often accompanied by at least some of their host's young in the nest (the cowbirds, *Molothrus* spp., parasitic finches, e.g. *Vidua* sp., and certain cuckoos and koels, e.g. *Clamator* spp.). Given the apparent benefits to parasites of being reared alone, it is unclear why nestmate-tolerance persists amongst these parasitic species. Some lineages may simply as yet lack the necessary genotypic variation on which selection can act (i.e. evolutionary lag, Davies 2000). Alternatively, disposing of one's nestmates might bring with it significant costs that maintain tolerance as the more adaptive strategy, despite the intrabrood competition it entails. Such possible costs include an increased risk of host abandonment for lone parasites in the nest (Broom et al. 2007); or costs to growth, either because killing is an energetically expensive activity (Anderson et al. 2009, Grim et al. 2009), or because the loss of assistance from nestmates in soliciting provisions ultimately results in a lower net food intake for the parasite (Kilner 2003, Kilner et al. 2004, Gloag et al. 2011).

A further, not mutually-exclusive, possibility is that in some parasites, nestmate-killing is not favoured for the same reason it is not favoured in non-parasitic species — nestlings share the nest with siblings. The hosts of certain nestmate-tolerant parasites are commonly observed to be multiply parasitized, receiving and rearing more than one parasite per brood, as well as some of their own young (Ortega 1998, Davies 2000). This multiple parasitism could arise solely from different females, or in part from the same female laying repeatedly in the same nest (hereafter: repeat parasitism). If the latter, and assuming a parasite chick cannot discriminate the siblings in their nest from other conspecifics or host young, those parasites that killed their nestmates would pay the indirect fitness cost of killing their kin.

From an adult female's perspective, repeat parasitism has the disadvantage of generating local competition between her offspring (Goguen et al. 2011), but may nevertheless be a good compromise if another suitable nest cannot be found. To date, parentage analyses of a handful of nestmate-tolerant parasites, from hosts in which some multiple parasitism occurs, have found both the same and different females may be responsible for eggs in a single nest (the great spotted cuckoo, *Clamator glandarius*, Martinez et al. 1998a; brown-headed cowbird, *Molothrus ater*, Alderson et al. 1999a, Hahn et al. 1999, McLaren et al. 2003; and bronzed cowbird *M. aeneus*, Ellison et al. 2006). The possibility of repeat parasitism has however not yet been estimated in those host-parasite pairs where we observe multiple parasitism to be truly commonplace.

In this study, we assess whether the same or different females are responsible for multiple parasitism by the nestmate-tolerant shiny cowbird (*M. bonariensis*) of a common host, the chalk-browed mockingbird (*Mimus saturninus*). High parasitism intensities in this host mean that a majority of cowbird eggs occur in multiply parasitized nests (Fraga 1985, Mason 1986, Sackmann and Reboreda 2003, Gloag et al. 2012). While some proportion of this multiple parasitism arises due to eggs laid the same day, and therefore can be assigned immediately to different females (at the site of this study, ≈ 20%), nests regularly attract parasitism over the period of several days while mockingbirds are themselves laying. Based on evidence from the distribution of eggs received per nest, there is no indication that cowbirds avoid laying in mockingbird nests that already contain cowbird eggs (Gloag et al. 2012). Nor is it necessarily true that cowbird nestlings suffer from the presence of other cowbirds in their nest, as chalk-browed mockingbirds, being large hosts (mean body mass 75 g vs. 45 g for cowbirds), are readily capable of rearing more than one cowbird chick in a brood (Fraga 1985).

If it occurs, repeat parasitism would also introduce an interesting dilemma for shiny cowbird females at the time of nest visits. Like most parasites, shiny cowbirds reduce their host's clutch prior to laying, in this case by puncturing holes in any pre-existing eggs with their beaks. Hosts later then remove the damaged eggs. Video recordings of cowbird visits to mockingbird nests show that, with very few exceptions, every egg-lay is preceded by a puncture attack (Gloag et al. 2012), and that mockingbird and cowbird eggs are attacked indiscriminately (Fiorini & Gloag, unpubl. data). Any female returning to a nest in which she had previously laid therefore must either selectively target only the eggs of mockingbirds or other cowbirds when puncturing, or risk destroying her own egg or eggs.

We determine the incidence of repeat parasitism by cowbirds directly, by filming egg-laying by individually-marked females, or when necessary, indirectly, via the comparison of egg morphs laid in the same nest. Shiny cowbird eggs are highly polymorphic in size, shape, background and spot colour, and particularly, spotting pattern (Friedmann 1929). The assumption that each individual shiny cowbird female lays eggs consistent in appearance was made in the first descriptions of multiply parasitized nests by Hudson (1874), and was the basis for Lyon (1997) and Kattan (1997) concluding that different females accounted for multiple eggs in nests of chestnut-capped blackbirds (*Agelaius ruficapillus*) and house wrens (*Troglodytes aedon*) respectively. Using sets of eggs from our marked females, we test this assumption for the first time and subsequently refer to egg morphology to assess repeat parasitism rates when female identity cannot be known from our video data.

2. METHODS

(a) *Trapping and marking*

This study was conducted in pampas grasslands at Reserva El Destino, Buenos Aires Province, Argentina (35°08′S, 57°23′W). We trapped 55 female cowbirds during spring–summer 2011, using walk-in funnel traps baited with millet. Previous trapping efforts at the site had suggested that females maintain home ranges within and between breeding seasons. We banded cowbirds with unique colour-ring combinations. Ring colours however can be difficult to distinguish in infra-red video recordings made in the low-light conditions of pre-dawn, when cowbird egg-laying occurs (Gloag et al. 2012). Thus, each cowbird was also given a unique head-mark by decolouring the distall end of head feathers with hair bleach to facilitate individual identification, as shown in Figure 1 and ESM1. We hereafter refer to these birds as ‘painted females’. Head-marks were readily identifiable and persisted for at least the 12 weeks of the study. In addition, cowbirds colour-ringed in previous years (but lacking head-marks) were active at the site. All manipulations were conducted under permit in accordance with Argentinian law.

Figure 1. ‘Painted’ females were individually marked by dying a unique pattern of the distal portion of head feathers blond, allowing identification of the female from infra-red nest-cam footage (example given in ESM1).

(b) ***Nest filming and monitoring***

We located mockingbird nests by searching trees within known breeding territories. Most egg-laying by cowbirds coincides with the mockingbird’s own laying period (mockingbird clutch size: 4-5 eggs, Fiorini et al. 2009). At 125 nests located during or prior to mockingbird laying, we suspended a ‘nest-cam’ (Handykam CCD colour microcamera) in the vegetation above the nest connected by cables to a digital video recorder (Lawmate PVR1000 or PVR500 ECO) at the base of the tree. Nests were filmed between twilight and dusk/late afternoon, each day until at least the end of the first day of the mockingbird's incubation period, or until the nest was predated or abandoned. Nests were considered abandoned if parents were not observed at the nest during the day's recording. Per nest, we were able to film the majority of cowbird laying visits (on average, 82% of parasitism), which occur prior to, or very shortly after, dawn, and also many puncturing ('non-laying') visits, which can occur anytime from sunrise to sunset. We checked the contents of nests at each day and labelled new cowbird eggs with permanent marker to allow individual identification. As a record of egg morph, each egg was photographed in the field using a Cannon SX230 camera against a common brown-cloth backpiece, away from strong light. On some occasions, we also added or removed eggs from nests during nest checks as part of a parallel experiment (Fiorini & Gloag, unpubl.), however we never removed eggs laid by painted females, allowing us to assess whether a female attacked her own egg if she made a return visit to the nest.

(c) ***Video analysis and egg morph assignment***

We assessed repeat parasitism rates by female shiny cowbirds based only on those nests that were active until at least the first day of incubation and were therefore likely to have attracted their full complement of parasitism (Fiorini et al. 2009). For each cowbird egg laid into a parasitized nest, we asked: 'Did the female that laid this egg previously lay in this nest?' Nest-cam footage was often sufficient to answer this question. However, in cases where the identity of the female that laid an egg could not otherwise be known (i.e, the female was not painted, or the laying event was not filmed), we compared the egg's morph relative to that of existing eggs in the nest to determine whether repeat parasitism could at least be excluded. Comparisons were made via a coarse-level assessment of egg spotting patterns. Five scorers naïve to the study's aims were asked to classify eggs from photographs into one of six categories, which represented variation in spotting pattern, using the panel shown in Figure 2. Morphological variation in cowbird egg spotting is better described as continuous, and these categories represent only arbitrary divisions that serve to discriminate between common morphs at our fieldsite. Scorers were presented with screen images of all eggs from nests in which an unidentified female laid, interspersed with eggs laid by painted females (700ppi resolution). We expected eggs of the same female to be consistently assigned to the same category within and between scorers. Following from this, two eggs that did not attract any scores in the same category, across all five scorers, were considered sufficiently different that the same female could not have laid them.

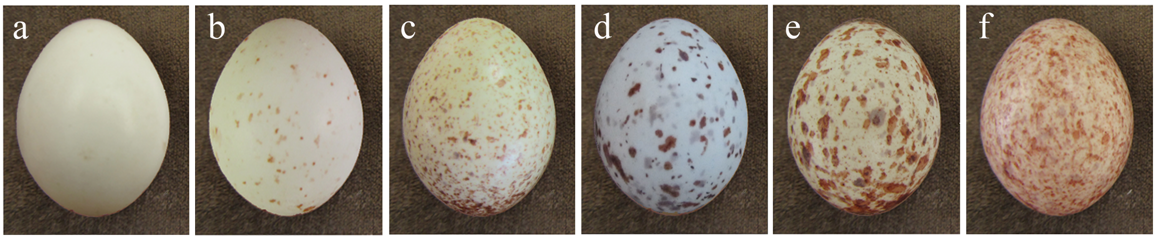
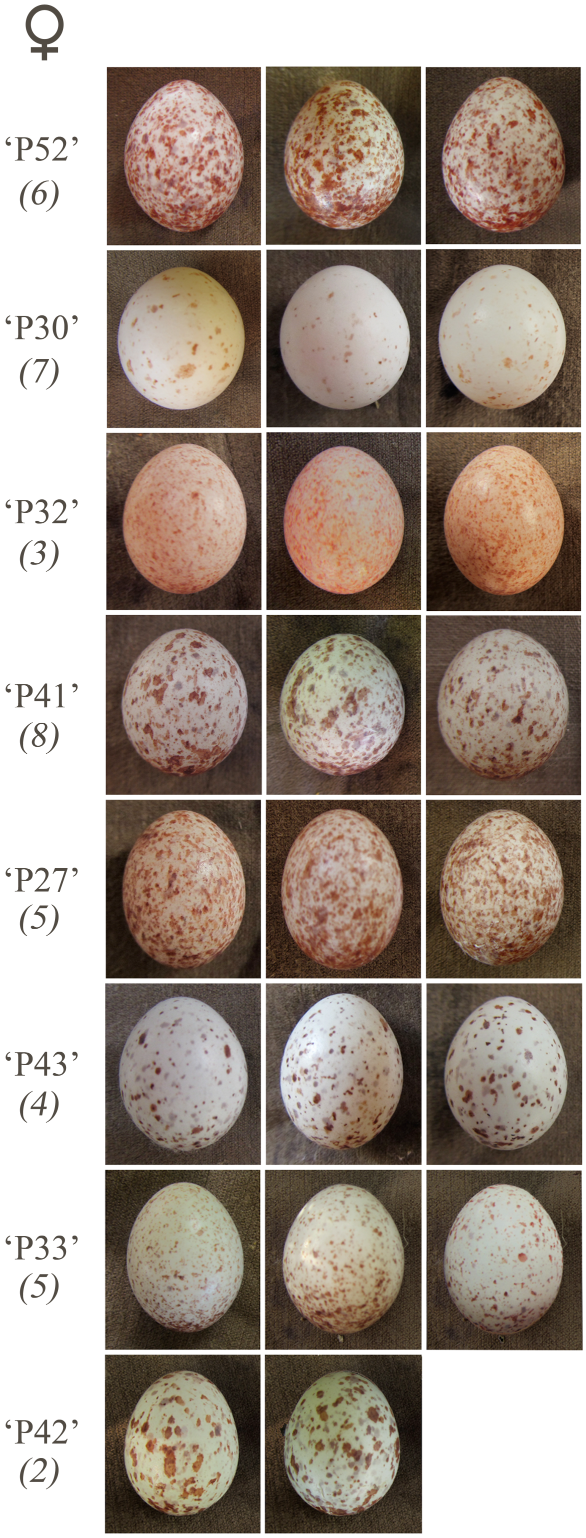


Figure 2. Six categories of egg morphs that span typical variation at our fieldsite. Where the identity of females could not be known from nest-cam footage, eggs were assigned to category by human scorers. The photos in this reference panel were slightly modified to give all eggs a common size and shape.

Figure 3. Examples of two or three eggs laid by each of eight painted shiny cowbird females on filmed laying visits to chalk-browed mockingbird nests; sample sizes of filmed egg-lays (in which egg assignment was unambiguous) for each female are given in parentheses. Eggs laid by the same female were very similar to the human eye in colour, spotting pattern, shape and size. When scored into our morph categories no two eggs by the same female were uniformly scored into different categories (see Figure 2 and main text).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'P52'
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(2)

3. RESULTS

(a) ***Within-individual consistency in egg morph***

We filmed 179 cowbird laying visits, including 67 by painted females of which 40 were the only cowbird egg laid in the nest that day and could thus be unambiguously assigned (see ESM1 for example footage). Eight females were represented in this sample, with 2-8 eggs per female. Eggs laid by the same female were very similar in appearance, as shown in Figure 3. When five scorers were asked to assign eggs to morph category (Figure 2), a painted female's eggs were uniformly assigned either into a single morph category (*n*=6 females) or one of two morph categories (*n*=2 females). In the latter cases, one or more eggs of the females attracted more than one category assignment between scorers. However, no two eggs laid by the same female were ever scored uniformly into different categories (that is, never satisfied our criterion for 'different females'), indicating our criterion for assessing eggs of unknown origin was sufficiently conservative.

(b) ***Estimate of repeat parasitism rate***

On no occasion was a painted female filmed returning to a nest she had already parasitized to lay again (0 of 67 laying visits, by 13 painted females), though painted females were filmed laying eggs on consecutive days in different nests (*n*=9 cases) and at 2-3 day intervals in different nests (*n*=12). Similarly, while painted females were filmed making puncturing visits to nests (*n*=54), we never observed them make such visits to active nests in which they had laid. We did twice film a puncturing visit to a recently abandoned nest in which the attacking female had already laid, and in both cases she attacked her own egg and in one instance successfully punctured it.

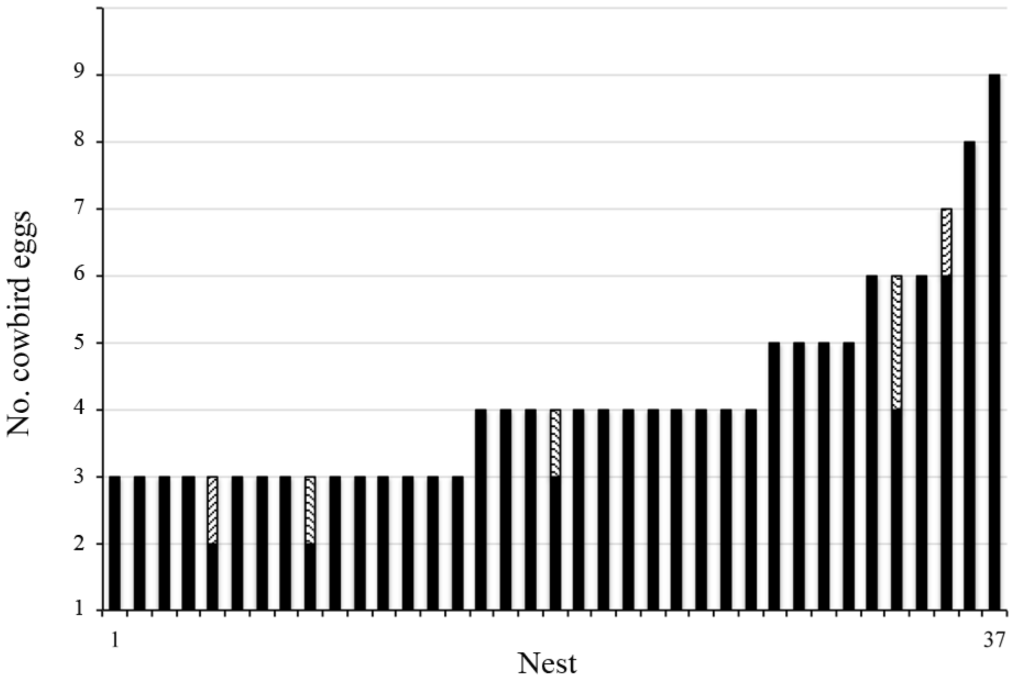

Figure 4. Cowbird eggs received by 37 multiply parasitized mockingbird nests that were filmed during the mockingbird's egg-laying period, and that remained active until at least the start of incubation. No eggs in the same nest were positively identified as laid by the same female. Black bars indicate the female that laid the egg had not previously parasitized the nest, based on video evidence or egg morph category. Hatched bars indicate equivocal cases, where it was not possible to confirm whether or not the female had previously parasitized the nest.

Fifty-two of our filmed nests remained active until the start of the incubation period or beyond, and were therefore considered to have attracted their full parasitism load. These nests received between one and eight cowbird eggs each (*n*=133 eggs in total, of which 118 were not the only egg laid in the nest, 89%); Figure 4. Of the 81 of these eggs laid into an already-parasitized nest, we could confirm that 74 were not repeat parasitism events (i.e. they were laid by a different female to any previous eggs in the nest based on video identification of females and/or egg morphology, 91%). This included all eggs laid in some nests of the upper quartile of parasitism intensities, with 5, 7 or 8 cowbird eggs in a nest each laid by a different female; Figure 4. For the remaining 7 eggs, we could neither confirm nor refute that an existing egg in the same nest had been laid by the same female, because females could not be differentiated using video data and eggs were not unanimously scored into different morph categories. If these cases all represented true repeat parasitism events, then at most 9% of eggs laid into parasitized nests were into nests in which the female had already laid (7/81). The actual proportion is likely less than this. Six of the seven cases involved a failure to differentiate between two eggs of the most common morph type (Figure 2, morph ‘e’, which accounted for 39% of all unidentified females’ eggs scored).

4. DISCUSSION

Based on direct identification of individual females from video recordings and indirect inference from egg morphs, we find that individual shiny cowbirds rarely, or possibly never, lay more than one egg per chalk-browed mockingbird nest. Rather, the high incidence of multiple parasitism in this host arises from the activity of multiple females, with as many as eight different females laying in the same nest. Thus while cowbird chicks in this host are likely to share the nest with one or more conspecific parasites (89% of all cowbird eggs occurred in multiply parasitized nests) they are unlikely to share with their maternal siblings. We find no evidence from this host therefore that indirect fitness costs are a major factor in maintaining young shiny cowbirds as nestmate-tolerant.

Our results are consistent with patterns of shiny cowbird nest-use proposed from other hosts (Kattan 1997, Lyon 1997). In those cases, eggs in the same nests were assigned to different females on the basis that they tended to look different in their colour or spottiness. Based on the confirmation of high within-individual similarity of eggs from this study, we show for the first time that egg morphology does provide a useful, non-invasive means to generate estimates of repeat parasitism by shiny cowbirds, though importantly, only upper-range estimates. That is, while eggs that look very different can be safely assumed to belong to different females, eggs that look very similar have not necessarily been laid by the same female.

By limiting themselves to just one egg per host brood, female cowbirds are obliged to make a greater time and energy investment in searching for and monitoring appropriate nests. Avoiding repeat parasitism presumably therefore also increases the cognitive ‘book-keeping’ demands on females (Nair-Roberts et al. 2006), which must maintain up-to-date spatial and temporal maps of active nests in their home ranges. Selection may have favoured this strategy however for a variety of reasons. Investing one egg per nest ensures that a female’s offspring do not compete with one another for food (Goguen et al. 2011) and dilutes the risk of predation or endoparasite infection (e.g. *Phylornis* sp.) for her clutch amongst the greatest possible number of nests (Hahn et al. 1999). Additionally, females that avoid revisiting nests avoid damaging their own eggs during puncture attacks. The latter holds also with respect to puncturing visits (i.e. non-laying visits), which we observed females making in the days prior to laying their eggs, but not after (with two exceptions, both at abandoned nests).

In the absence of repeat parasitism by females, a young shiny cowbird might nevertheless share the nest with kin if females mated to the same male laid in the same nest. The mating system of shiny cowbirds is unknown, but Friedmann (1929), observing numerous pairings of ringed individuals, proposed that both sexes were largely monogamous. This would be in keeping with evidence from some other brood parasites, which found monogamy, while not always strict, to be the prevalent mating system (Marchetti et al. 1998, Martinez et al. 1998b, Langmore et al. 2007), including in the shiny cowbird's nearest relative, the brown-headed cowbird (Alderson et al. 1999b, Strausberger and Ashley 2003). Even assuming highly promiscuous mating by shiny cowbird males, shared paternity in nestmates would depend on females mated to the same male independently locating and targeting the same host nest, and therefore is unlikely to generate high incidences of kinship within nests.

We suggest however that, even where parasites sharing a nest are not kin, multiple parasitism may have important consequences for the evolution of nestling virulence. Multiple parasitism necessarily alters the typical competitive environment of the brood and might therefore increase or decrease the selective pressures on parasites to eliminate nestmate competition. The direction of this selective pressure is not always easy to predict. For example, for shiny cowbirds parasitizing mockingbirds and other large hosts, multiply parasitized clutches could, on the one hand, lead to larger brood sizes, and thus an increase in intrabrood competition and the corresponding costs to parasites of sharing the nest. On the other hand, if females commonly break at least one host egg for each of their own laid, but rarely break the thick-shelled eggs of other cowbirds (Tuero et al. 2012, Gloag et al. 2012), then multiple parasitism may often lead to large, competitive host nestmates being replaced with less competitive, conspecific nestmates, and ultimately reduce the costs for any one parasite nestling of sharing a nest, relative to a parasite in a singly parasitized brood. Particularly in generalist parasites, such as shiny cowbirds, where selection on virulent behaviours might be acting on the average benefit of such behaviours across many hosts (Gloag et al. 2011), these shifts in brood competitiveness caused by multiple parasitism may be significant, and remain to be investigated.

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Chapter 7

General Discussion

General Discussion

In the opening chapter of this thesis, I proposed that the goals of my research were two-fold: (i) to address certain puzzles of avian brood parasitism – particularly in the context of generalism of host use by parasites and multiple parasitism of host nests, these being defining features of shiny cowbird parasitism – and (ii) to advance our basic knowledge of the biology of the shiny cowbird system. This final chapter provides a brief summary of the thesis findings, in light of these aims and some of the many questions that remain for future investigation.

1. Contributions to some puzzles

Why don't hosts simply remove parasite eggs from their nests?

Broadly speaking, there are two explanations for why a host species does not perform a particular defence behaviour: either they should do so, but don't (that is, the defence would be adaptive under current conditions), or they don't do so because they shouldn't (that is, despite first appearances, the defence would not in fact be adaptive under current conditions). In the case of hosts rejecting parasite eggs, it is the former line of reasoning that has attracted the most empirical support: thus we accept that many hosts would enjoy a net benefit from rejecting the eggs of a parasite, but they are fooled into accepting them due to some property of the egg (e.g. mimicry or crypsis; Langmore et al. 2008, Stoddard and Stevens 2011). Support for the alternative explanation meanwhile, asks that we identify one or more significant costs associated with egg rejection, relatively few of which have been documented to date (Davies et al. 1996, Hoover and Robinson 2007). Chapter 2 adds to this short list by illustrating there is a cost of removing parasite eggs from the clutch for hosts that are multiply parasitized. In this case, egg acceptors benefit relative to egg rejecters because previously-laid parasite eggs reduce the risk that host eggs are destroyed by later parasites that visit the nest (Sato et al. 2010), a ‘dilution effect’ that we show holds true for mockingbirds parasitized by shiny cowbirds.

It remains to be established whether the clutch dilution effect can ultimately lead to a net benefit of accepting parasite eggs for mockingbirds or any other multiply parasitized host. Whatever the outcome however, our findings are an important illustration that the costs and benefits to hosts of anti-parasite defences may critically depend not only on the incidence of parasitism in the host population (e.g Davies et al. 1996), but also the intensity. Multiple parasitism clearly has a role to play in shaping host defences, and will, I hope, attract further research attention.

Is the only goal of front-line anti-parasite defences to prevent parasitism itself?

On showing others the videos of mockingbirds mobbing cowbirds in their nests, one of the most common questions I am asked is: why do they bother? After all, such vigorous battles may attract unwanted attention to the nest (including other parasites) and do very little to prevent parasitism occurring. Chapter 3 presents one answer to this query, showing that mobbing in the nest reduces the success of cowbird puncture attacks even where those cowbirds go on to lay. To my knowledge, this is the first evidence that mobbing is not exclusively concerned with preventing parasites from laying eggs.

Presently, the interactions between hosts and parasites at the front-line are less well understood than those at later stages in the nesting cycle, but I suspect this knowledge gap will close in coming years as the use of remote cameras becomes widespread, and research interest is stimulated by recent works that show an important role for front-line interactions in host-parasite evolution (Davies and Welbergen 2008, Guigueno and Sealy 2011, Feeney et al. 2012, Thorogood and Davies 2012). For mockingbirds and shiny cowbirds, we are only just beginning to shed light on the battle that precedes the cowbird laying its egg, and our catalogue of 'nest-cam' videos introduce various new questions. What, for example, are the costs of mobbing in terms of attracting predators or other cowbirds to the nest? Do female cowbirds have physiological or morphological adaptations that allow them to withstand mobbing? And what causes some females to arrive at host nests more or less synchronously with other females? The latter is a particularly intriguing observation, for which I earlier proposed opportunism as the most likely explanation (see Chapter 3) on the basis that second-to-arrive females may be able to approach the nest more freely when hosts are distracted. It would be interesting however to know whether females that arrived together to parasitize nests also foraged and searched for nests together (suggesting not opportunism but, perhaps, cooperation?), or whether second-to-arrive females had never previously located the nest (suggesting eavesdropping?). I am hopeful that advances in animal-tracking technology may allow us to make headway on such questions in the near future.

Do differences between host and parasite nestling begging signals help or hinder the parasite in securing host care?

To the human observer, the more dissimilar are parasites to their host's young, the more perplexing we find the host parent's acceptance of them. Yet in some cases parasites rely on these very differences to secure the necessary level of host care; for example, a larger relative size can allow parasites to monopolize provisions over their host nestmates (Lichtenstein and Sealy 1998). Chapter 4 reveals that differing from the host young's phenotype can also benefit parasites with respect to the acoustic structure of their begging calls. Shiny cowbird calls differ greatly from those of same-age house wrens, yet when both call types were broadcast at unparasitized wren broods, at a fixed call rate and amplitude, it was the cowbird's call that provoked the greater provisioning effort from parents. Further experiments indicated that the long tremulous structure of the cowbird's call exploits parents' sensitivity to an increase in the brood's call rate (or perhaps more simply, the brood's output of sound energy). This provisioning rule is not specific to house wrens, but likely widespread among birds (Wright and Leonard 2002), and indeed, we found the same pattern of provisioning responses when experiments were replicated in the great tit, even though cowbirds' begging signals clearly did not evolve to exploit this allopatric species.

The use of playback experiments to evaluate call structure has its drawbacks. Begging is, after all, a complex and integrated visual and vocal signal, and we must assume that parent's responses during broadcasts represent well those they would display when faced with a real parasite in the nest. On the other hand, playback experiments allow us great control over which parameters of a call we investigate, particularly as we can present parents with calls altered to adjust particular acoustic properties (such as in the case of the 'truncated' or 'clustered' calls we used). Playback experiments are sure then to have a role in future efforts to unravel the ways in which parasite's manipulate their hosts via their call's structure, and so shed light on why selection has favoured vocal mimicry in some parasites but not others.

I should note that I have presented the playback experiments of Chapter 4 out of their true chronological order for the sake of a clear presentation. In fact, I began with an experiment (largely opportunistic) in great tits, and later replicated it in house wrens to confirm the result's relevance in a true cowbird host. A valuable next step would be to replicate these experiments once more in a second common cowbird host, preferably one whose calls differ greatly in structure from those of all three previous subjects – house wren, great tit, and cowbird. This would serve as a necessary test of the notion that a tremulous call structure is largely 'universal' in its effectiveness, and thus of particular benefit to an extreme generalist parasite.

Why don't all nestling parasites kill their host's offspring?

Chapter 5 presented a mathematical model that formalized the conditions under which either nestmate-killing or nestmate-tolerance will be adaptive for parasites, assuming that selection favours the strategy that brings the parasite the highest provision intake (Kilner 2005). Nestmate-killing, we find, will not be adaptive in all cases. Nestmate-tolerance will be favoured wherever parasites are worse than host young at stimulating parents to bring food to the nest, better than host young at competing for food when it arrives, or both.

What can be considered the adaptive strategy for generalist parasites, however, is not easily assessed. Many generalist parasites are likely to have different relative abilities to solicit and compete for provisions in different hosts (including brown-headed cowbirds, for whom the notion of nestmate-tolerance as adaptive was first proposed, Kilner et al. 2004). Certainly the situation is complex for shiny cowbirds. In the field we found that nestling cowbirds benefitted from the presence of host chicks when parasitizing house wrens, but not when parasitizing mockingbirds. Evaluating how selection for virulent behaviours might act on a parasite population using dozens of hosts, each of which offers a different magnitude of pay-off for nestmate-killing, will be a task for future theoretical work.

Meanwhile, ripe for investigation in the field is the effect of multiple parasitism on the competitive environment that parasite's face in the brood. From our model, it is clear that multiple parasitism will add a further dynamic to the provision's trade-off, as it shifts the relative parameter values of a parasite's nestmates. A second possible way in which multiple parasitism might influence selection on nestmate-killing behaviours can, however, probably be ruled out for shiny cowbirds, based on the results of Chapter 6. If female parasites have a habit of laying more than once in the same host nest, young parasites might regularly share the nest with their siblings, thereby favouring tolerance towards nestmates as a means to increase inclusive fitness. At least when parasitizing mockingbirds, all present evidence suggests that female shiny cowbirds lay just one egg per nest.

2. Shiny cowbird parasitism: what we do and don't know

We know more about shiny cowbird behaviour now than when work for this thesis began, in large part thanks to the video recordings made at mockingbird nests. These recordings both confirmed previous hypotheses about aspects of cowbird behaviour (females lay just one egg per nest, puncture attacks are not made after laying, females lay eggs of a consistent morph) and revealed some genuine surprises (cowbirds' ability to withstand mobbing, the 'frenzied' nature of puncture attacks, and the synchrony of laying visits by some females).

Four further observations that did not find a place in preceding chapters are worth adding here. Firstly, we filmed 8 cases of cowbirds parasitizing house wren nestboxes. Wrens were never observed to mob the cowbird, but if present, left the box shortly before the parasite entered; an example is given in ESM 1. Secondly, one filmed case of parasitism of a house wren was by a painted female that, ten days later, was also filmed parasitizing a nearby mockingbird nest (see ESM 2). This supports the idea that individual females are not host-specialists, but of course for the moment it remains an isolated observation. Thirdly, although male shiny cowbirds in captivity exhibit puncturing behaviour when presented with eggs (Llambías et al. 2006), we never observed male shiny cowbirds making puncturing visits at mockingbird or wren nests. Nor were males seen to assist a female's nest access by distracting hosts, as has been reported in screaming cowbirds (Fraga 1998, *pers. obs.*) and some cuckoos (Davies 2000). Finally, despite some evidence from studies with captive cowbirds to the contrary (Tuero et al. 2011), we found that cowbirds showed no egg type bias during puncture attacks in mockingbird nests. This finding is implied by the data presented in Chapter 2, but a full analysis will form part of a manuscript co-authored with V. Fiorini, currently in preparation. We hypothesized that females might assist their offspring by preferentially targeting mockingbird eggs (as mockingbird nestmates will be stronger competitors than conspecific nestmates) but this was not confirmed. Perhaps selection for such a preference is weak because females approach the same desirable outcome by striking at all eggs in a clutch indiscriminately, mockingbird eggs being the most likely to break when struck.

Needless to say, many gaps remain in our knowledge of shiny cowbird biology. In my view, particularly pressing is to elucidate the patterns of host use by individual shiny cowbird females, and the cowbird's mating system. With this information, we will be in a better position to predict how selective pressures may have acted on cowbird's behaviours, and to model the evolutionary dynamics of cowbird-host interactions. Beyond this, we might begin to quantify and compare cowbirds' interactions with some of their hundred or so other common hosts, very few of which have been studied in any way.

3. Concluding remarks

To conclude with a reflection: When I started this thesis I was principally aware of the biology of the common cuckoo and its hosts, and so approached my own study system with a set of what I now see were preconceptions, or even prejudices. As time progressed however, the diversity of the avian brood parasite systems became ever more apparent; general biological principles can guide us from one system to another but, in the end, there is no shortcut to replace the in-depth study of each in the field. In doing so, we find that each system will offer its own particular insights into the behavioural ecology and evolution of brood parasitism. I hope my work has gone some way to show that this holds true for shiny cowbirds and their hosts, and that I have made clearer a path for future research with these birds.

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