

Brood parasite eggs enhance egg survivorship in a multiply parasitized host

Ros Gloag^{1,*}, Vanina D. Fiorini², Juan C. Rebores²
and Alex Kacelnik¹

¹Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

²Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II Ciudad Universitaria, C1428EGA Buenos Aires, Argentina

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, counterintuitively, high intensities of parasitism might decrease or even reverse selection pressure for host defence via egg rejection.

Keywords: host defence; evolutionary equilibrium; egg rejection; risk dilution; *Molothrus bonariensis*; *Mimus saturninus*

1. INTRODUCTION

Some hosts of brood parasitic birds recognize and remove alien eggs from their nests [1,2]. Those that do not might later reject the parasite nestlings directly or abandon the breeding attempt [3,4]. 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 [2]. One line of argument invokes a stepwise coevolutionary arms race between brood parasites and their hosts in which host defences periodically ‘lag’ behind the adaptations of the parasite [5–7], evidenced, for example, in the remarkable egg mimicry evolved by some cuckoos to impede their host’s recognition of foreign eggs [8,9]. 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 [10–12]. A few such costs have been identified; hosts might break their own eggs when attempting to reject a foreign egg [13,14], erroneously reject their own eggs [15,16], encounter physical constraints (coupled with low re-nesting success [17]) or endure retaliatory nest predation by ‘mafia’ parasites [18,19].

In this study, we report a novel cost of egg rejection that arises when hosts are multiply parasitized [20]. Multiple

parasitism, where host nests receive two or more parasite eggs, is common to many avian host–parasite systems [21]. 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.* [20] 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 counterintuitive scenario in which high intensities of parasitism select against the evolution of defensive egg rejection, rather than the reverse [16,22–25].

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: 70–75 and 40–50 g, respectively) endure high incidences and intensities of parasitism [26], and accept nearly all cowbird eggs despite these being different in size, shape and colour to their own [27,28] (figure 1). Meanwhile, shiny cowbird females 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 in the electronic supplementary material, videos S1–S3). Thus, while mockingbird nestlings fare well in parasitized

* Author for correspondence (ros.gloag@zoo.ox.ac.uk).

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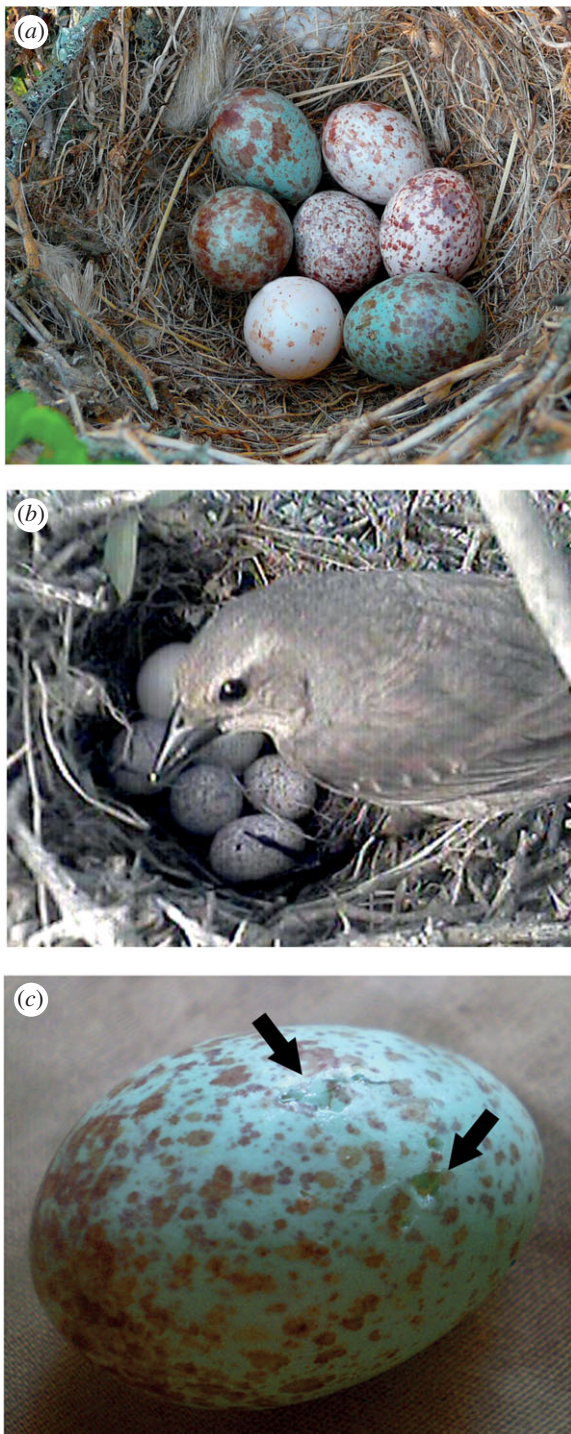


Figure 1. (a) A chalk-browed mockingbird nest multiply parasitized by shiny cowbirds (smaller, pale-background eggs). (b) A shiny cowbird puncturing eggs in the nest of a mockingbird that already contains cowbird eggs. (c) A mockingbird egg broken during a cowbird attack, with arrows to mark where the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

broods (hatching and fledging at rates akin to those from unparasitized broods [29,30]), egg losses suffered owing to parasitism can be severe [31]. 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 then expect

more mockingbird eggs to survive cowbird puncture attack 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 2010–2011 season. Most cowbird laying is synchronized with the mockingbird's own laying period (one egg per day, clutch size 4–5 [32]), 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 04.30 to 10.00 h 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, whereas some or all cowbird eggs were allocated to other projects.

(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 that would affect the pay-offs of clutch dilution), we used a χ^2 goodness-of-fit test of the observed distribution of parasite egg load per nest ($1 - \text{CDF}$) against the expected values under a geometric probability distribution (i.e. the expected distribution if parasitism events occur independently [33]).

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 §3 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, seconds) 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 electronic supplementary material, videos S2–S3, and §3 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 behaviour 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 χ^2 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 [34]. 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 with 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 towards one or the other egg type [35], 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 [36–41].

Confidence intervals (95%) of proportions were calculated by the exact method [42]. All statistical tests were performed in SPSS STATISTICS v. 17.0 or MATLAB v. 7.10.0 (R2010a).

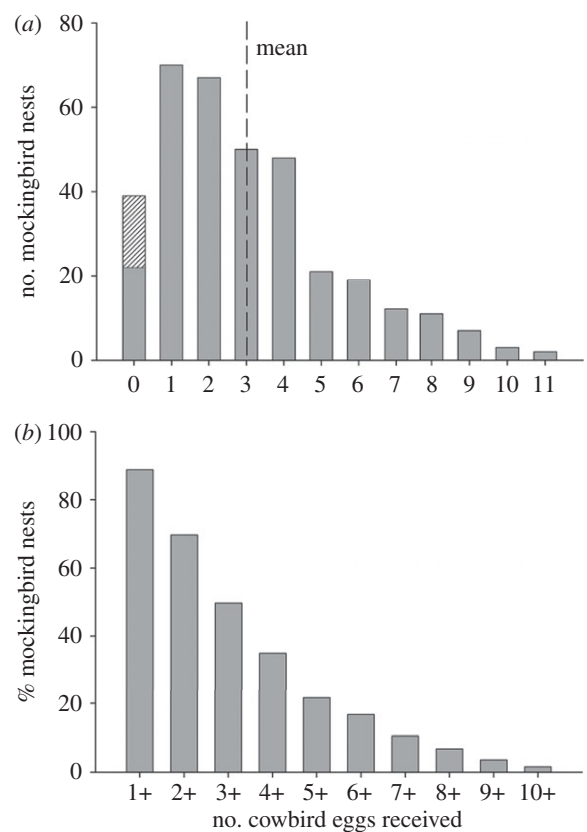


Figure 2. (a) The number of cowbird eggs found in mockingbird nests at our field site during 2008–2011 ($n = 347$ nests); some nests that were not parasitized nevertheless lost eggs to cowbird puncturing (striped bar). The dotted line indicates the mean. (b) For the same data, the distribution of nests 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.

3. RESULTS

(a) Parasitism intensity

Of all nests ($n = 347$), 89 per cent were parasitized with one or more cowbird eggs and a further 5 per cent 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 (\pm 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 (χ^2 goodness of fit test for a geometric distribution, $\chi^2 = 12.2$, 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 (electronic supplementary material, video S3). During laying visits

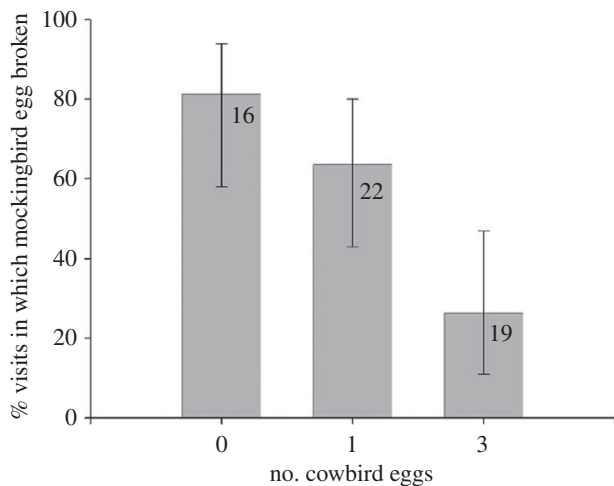


Figure 3. Proportion of cowbird visits (with 95% CI) that resulted in mockingbird eggs being broken when the mockingbird egg was the only egg in the nest, or when accompanied by one or three cowbird eggs at the time of puncture attack. Sample sizes for each treatment group are given inside the bars.

(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 that were abandoned the day of the recording; e.g. electronic supplementary material, videos S2 and S3). While the mean duration of puncture attacks was lower for mobbed visits (mean \pm s.e., not mobbed: 6.7 ± 1 s, mobbed: 3 ± 0.2 s, *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, χ^2 test of independence: $\chi^2 = 0.3$, d.f. = 1, $p = 0.6$). Furthermore, just 5 per cent of puncturing (post-sunrise) visits were mobbed (one of 21 to active nests), indicating cowbirds can bypass mockingbird nest defence entirely for such visits (e.g. electronic supplementary material, video S1).

During puncture attacks, cowbirds struck at both mockingbird and cowbird eggs (e.g. electronic supplementary material, videos S1–S3). 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 with 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 per cent 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 \times 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 per cent 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 ($\chi^2 = 11.5$, 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 ($\chi^2 = 0.7$, d.f. = 2, $p = 0.96$) or parasitism status at the time of the visit ($\chi^2 = 0.1$, d.f. = 1, $p = 0.75$).

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 four of 22 visits when one cowbird egg was present, and one or more eggs broken in six of 19 visits when three cowbird eggs were present; Fisher's exact tests: $p = 0.03$ and 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 [43–46]. 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 10 000 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

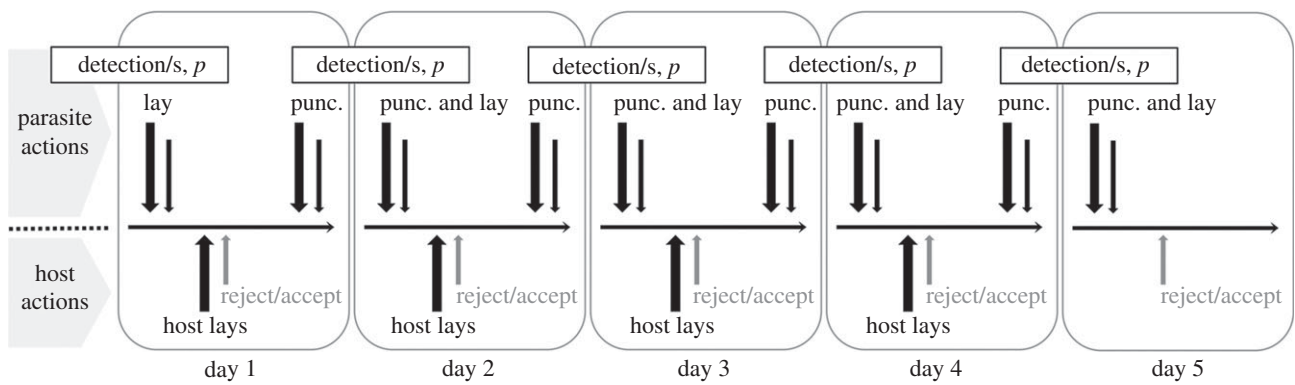


Figure 4. A simulation of multiple parasitism at host nests. Parasite actions are given in the top half of the diagram. Each parasite that detects a nest makes a puncturing visit and a laying visit the following morning during which both puncturing and laying occur. The puncturing visits that precede 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.

from retaining fewer of one's own eggs, whereas 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 less than 0.01. The MATLAB script and a glossary of terms are provided in the electronic supplementary material, S4 and S5.

The simulation makes the following assumptions and simplifications:

- 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 [32]. 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.
- 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 $a = 5$ that approximates the ratio between double and single parasitism per day we observed in mockingbird nests from our field study (18 of 87 nest recordings).
- 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.
- 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 towards 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

$$d_H = \frac{1}{H} \times \left(\frac{H}{H + P \times b} \right) = \frac{1}{H + P \times b} \quad (4.1)$$

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

$$d_P = \frac{1}{P} \times \left(\frac{P \times b}{H + P \times b} \right) = \frac{1}{(H/b) + P}. \quad (4.2)$$

- 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 [36–40]. 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.

(b) Egg survival: acceptors versus 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, $s_P = 0.3$).

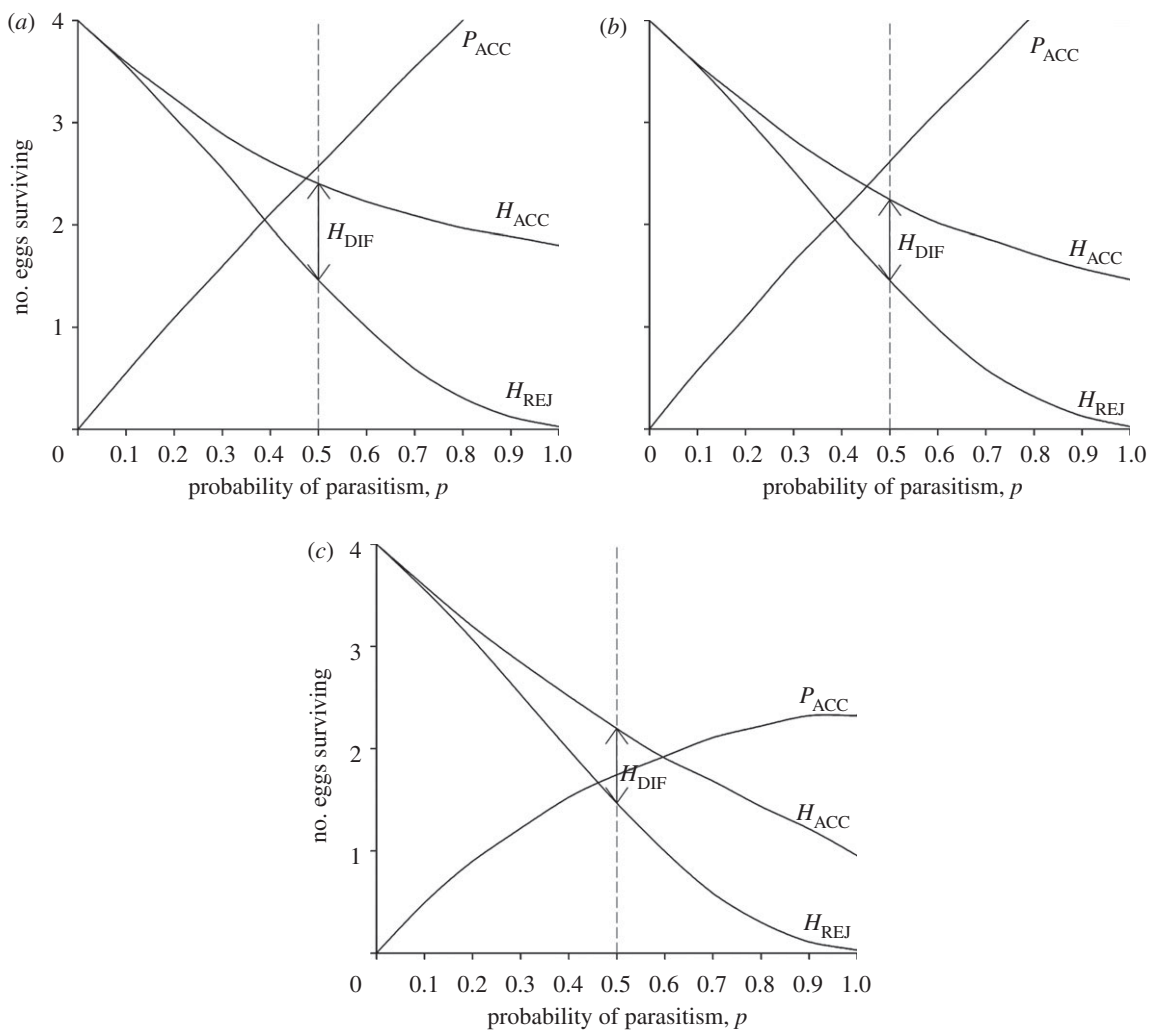


Figure 5. The consequences to egg survivorship of rejection or acceptance of parasite eggs by hosts for increasing probabilities of parasitism (p), the mean number of host eggs surviving for acceptors (H_{ACC}), rejecters (H_{REJ}), the difference between which is (H_{DIF}) and the mean number of parasite 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, $s_H = 0.9$, $s_P = 0.3$, $b = 0.7$; (c) all eggs equally likely to be attacked and broken if attacked, $s_H = 0.9$, $s_P = 0.9$, $b = 1$.

A p -value of around 0.5 approximates the parasitism intensity endured by mockingbirds at our field site, corresponding to a mean (\pm s.d.) 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 electronic supplementary material, S6 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) the 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 the *Vidua* finches [21], non-evicting cuckoos [47] and the South and Central American cowbirds. In the case of cowbirds, Ortega & Ortega [48] summarize reports of parasitism intensities and suggest that multiple parasitism may be the rule for at least some hosts of each of the bronzed cowbird (*Molothrus aeneus*), giant cowbird (*Molothrus oryzivorus*), screaming cowbird (*Molothrus rufoaxillaris*) and shiny cowbird, with the latter appearing to have the highest scores. Parasitism by cowbirds may also be particularly damaging at the egg stage because, unlike some other brood parasites, they commonly make both pre-laying visits and laying visits and may attack eggs on both [48,49].

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 [50]). Clearly, where parasite eggs greatly compromise the survival of host young, no amount of clutch dilution will tip the balance in favour of egg acceptance. This includes any negative effects that parasite eggs may have on host egg incubation and hatchability [51,52]. However, the mortality of host offspring in parasitized broods varies greatly between cowbird hosts [48,53], and hosts of brood parasites generally [21]. 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 nest-mates [54,55], 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 [4,20]. In the case of mockingbirds, host nestlings fare well in parasitized nests, hatching at the same time or one day later than their cowbird nest-mates, receiving an equal or greater share of provisioning and growing larger than them within a few days [56], to the extent that their hatching and fledging successes are similar to those of unparasitized broods [29,30].

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 [50,57]. 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 when compared with their parasites, because smaller hosts must work harder to rear parasite chicks. Even so, several studies on small-bodied hosts of brown-headed cowbirds (*Molothrus ater*) have failed to detect an effect of parasitism on parents' subsequent survival and nesting success [51,58–60] 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 [27] 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. 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 [28], a morph laid infrequently in their nests (less than 10%; R.G., 2010, unpublished data), while accepting all other eggs. Sackmann & Reboreda [29] 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, then 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 such as mockingbirds, white eggs may, for example, increase nest detectability by predators [61]. 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 the hosts of cowbirds and other 'nest-mate-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 [16,22–25]. Interestingly, the clutch dilution hypothesis contradicts this intuitive expectation. 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.

All manipulations were performed under permit according to Argentinian regulations.

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REFERENCES

- Rothstein, S. I. 1990 A model system for coevolution: avian brood parasitism. *Annu. Rev. Ecol. Syst.* **21**, 481–508. (doi:10.1146/annurev.es.21.110190.002405)
- Winfrey, R. 1999 Cuckoos, cowbirds and the persistence of brood parasitism. *Trends Ecol. Evol.* **14**, 338–343. (doi:10.1016/S0169-5347(99)01643-2)
- Langmore, N. E., Hunt, S. & Kilner, R. M. 2003 Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422**, 157–160. (doi:10.1038/nature01460)
- Sato, T., Tokue, K., Noske, R. A., Mikami, O. K. & Ueda, K. 2010 Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. *Biol. Lett.* **6**, 67–69. (doi:10.1098/rsbl.2009.0540)
- Dawkins, R. & Krebs, J. R. 1979 Arms races between and within species. *Proc. R. Soc. Lond. B* **205**, 489–511. (doi:10.1098/rspb.1979.0081)
- Davies, N. B. 1999 Cuckoos and cowbirds versus hosts: co-evolutionary lag and equilibrium. *Ostrich* **70**, 71–79. (doi:10.1080/00306525.1999.9639751)
- Langmore, N. E., Stevens, M., Maurer, G. & Kilner, R. M. 2009 Are dark cuckoo eggs cryptic in host nests? *Anim. Behav.* **78**, 461–468. (doi:10.1016/j.anbehav.2009.06.003)
- Stoddard, M. C. & Stevens, M. 2011 Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution* **65**, 1–9. (doi:10.1111/j.1558-5646.2011.01262.x)
- Spottiswoode, C. N. & Stevens, M. 2011 How to evade a coevolving brood parasite: egg discrimination versus egg variability as host defences. *Proc. R. Soc. B* **278**, 3566–3573. (doi:10.1098/rspb.2011.0401)
- Takasu, F. 2010 Why do all host species not show defence against avian brood parasitism: evolutionary lag or equilibrium? *Am. Nat.* **151**, 193–205. (doi:10.1086/286111)
- Lotem, A. & Nakamura, H. 1998 Evolutionary equilibria in avian brood parasitism: an alternative to the 'arms race-evolutionary lag' concept. In *Parasitic birds and their hosts: studies in coevolution* (eds S. I. Rothstein & S. K. Robinson), pp. 223–235. Oxford, UK: Oxford University Press.
- Kruger, O. 2007 Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Phil. Trans. R. Soc. B* **362**, 1873–1886. (doi:10.1098/rstb.2006.1849)
- Rohwer, S., Spaw, C. D. & Roskaft, E. 1989 Costs to Northern orioles of puncture-ejecting parasitic cowbird eggs from their nests. *Auk* **106**, 734–738.
- Roskaft, E., Rohwer, S. & Spaw, C. D. 1993 Cost of puncture ejection compared with costs of rearing cowbird chicks for northern orioles. *Ornis Scand.* **24**, 28–32. (doi:10.2307/3676406)
- Lotem, A., Nakamura, H. & Zahavi, A. 1995 Constraints on egg discrimination and cuckoo host coevolution. *Anim. Behav.* **49**, 1185–1209. (doi:10.1006/anbe.1995.0152)
- Davies, N. B., Brooke, M. D. & Kacelnik, A. 1996 Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proc. R. Soc. Lond. B* **263**, 925–931. (doi:10.1098/rspb.1996.0137)
- Kruger, O. 2011 Brood parasitism selects for no defence in a cuckoo host. *Proc. R. Soc. B* **278**, 2777–2783. (doi:10.1098/rspb.2010.2629)
- Soler, M., Soler, J. J., Martinez, J. G. & Moller, A. P. 1995 Magpie host manipulation by great spotted cuckoos: evidence for an avian Mafia? *Evolution* **49**, 770–775. (doi:10.2307/2410329)
- Hoover, J. & Robinson, S. 2007 Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. *Proc. Natl Acad. Sci. USA* **104**, 4479–4483. (doi:10.1073/pnas.0609710104)
- Sato, N. J., Mikami, O. K. & Ueda, K. 2010 The egg dilution effect hypothesis: a condition under which parasitic nestling ejection behaviour will evolve. *Ornithol. Sci.* **9**, 115–121. (doi:10.2326/osj.9.115)
- Davies, N. B. 2000 *Cuckoos, cowbirds and other cheats*. London, UK: Princeton University Press.
- Rothstein, S. I. 1975 An experimental and teleonomic investigation of avian brood parasitism. *Condor* **77**, 250–271. (doi:10.2307/1366221)
- Davies, N. B. & Brooke, M. L. 1989 An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts II. Host egg markings, chick discrimination and general discussion. *J. Anim. Ecol.* **58**, 225–236. (doi:10.2307/4996)
- Marchetti, K. 1992 Costs to host defence and the persistence of parasitic cuckoos. *Proc. R. Soc. Lond. B* **248**, 41–45. (doi:10.1098/rspb.1992.0040)
- Takasu, F., Kawasaki, K., Nakamura, H., Cohen, J. & Shigesada, N. 1993 Modeling the population dynamics of the cuckoo–host association and the evolution of host defences. *Am. Nat.* **142**, 819–839. (doi:10.1086/285574)
- De Marsico, M., Mahler, B., Chomnalez, M., Di Giacomo, A. & Reboreda, J. 2010 Host use by generalist and specialist brood parasitic cowbirds at population and individual levels. *Adv. Stud. Behav.* **42**, 81–119. (doi:10.1016/S0065-3454(10)42003-3)
- Fraga, R. M. 1985 Host–parasite interactions between chalk-browed mockingbirds and shiny cowbirds. *Ornithol. Monogr.* **36**, 829–844.
- Mason, P. 1986 Brood parasitism in a host generalist the shiny cowbird *Molothrus bonariensis* II. Host selection. *Auk* **103**, 61–69.
- Sackmann, P. & Reboreda, J. C. 2003 A comparative study of shiny cowbird parasitism of two large hosts, the chalk-browed mockingbird and the rufous-bellied thrush. *Condor* **105**, 728–736. (doi:10.1650/7194)

- 30 Fiorini, V. 2007 Synchronization of parasitism and host selection in a generalist brood parasite, the shiny cowbird. *Molothrus bonariensis* (*Icterinae*, *Aves*). PhD thesis, University of Buenos Aires, Argentina.
- 31 Salvador, S. A. 1984 Study of parasitism in raising shiny cowbirds *Molothrus bonariensis* and chalk-browed mockingbirds *Mimus saturninus* in Villa Maria Cordoba, Argentina. *Hornero* **12**, 141–149.
- 32 Fiorini, V. D., Tuero, D. T. & Reboreda, J. C. 2009 Shiny cowbirds synchronize parasitism with host laying and puncture host eggs according to host characteristics. *Anim. Behav.* **77**, 561–568. (doi:10.1016/j.anbehav.2008.11.025)
- 33 Snedecor, G. W. & Cochran, W. G. 1989 Statistical methods, pp. 75–79, 8th edn. Ames, IA: Blackwell Publishing.
- 34 Peeters, E. H. M. & Gardeniers, J. J. P. 1998 Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwat. Biol.* **39**, 605–615. (doi:10.1046/j.1365-2427.1998.00304.x)
- 35 Llambias, P. E., Ferretti, V. & Reboreda, J. C. 2006 Egg discrimination and sex-specific pecking behaviour in parasitic cowbirds. *Ethology* **112**, 1128–1135. (doi:10.1111/j.1439-0310.2006.01272.x)
- 36 Spaw, C. D. & Rohwer, S. 1987 A comparative study of eggshell thickness in cowbirds and other passerines. *Condor* **89**, 307–318. (doi:10.2307/1368483)
- 37 Picman, J. 1989 Mechanism of increased puncture resistance of eggs of brown-headed cowbirds. *Auk* **106**, 577–583.
- 38 Brooker, M. G. & Brooker, L. C. 1991 Eggshell strength in cuckoos and cowbirds. *Ibis* **133**, 406–413. (doi:10.1111/j.1474-919X.1991.tb04589.x)
- 39 Mermoz, M. E. & Ornelas, J. F. 2004 Phylogenetic analysis of life-history adaptations in parasitic cowbirds. *Behav. Ecol.* **15**, 109–119. (doi:10.1093/beheco/arg102)
- 40 Rahn, H., Curran-Everett, L. & Booth, D. T. 1988 Eggshell differences between parasitic and nonparasitic Icteridae. *Condor* **90**, 962–964. (doi:10.2307/1368865)
- 41 Igic, B., Braganza, K., Hyland, M. M., Silyn-Roberts, H., Cassey, P., Grim, T., Rutila, J., Moskat, C. & Hauber, M. E. 2011 Alternative mechanisms of increased eggshell hardness of avian brood parasites relative to host species. *J. R. Soc. Interface* **8**, 1654–1664. (doi:10.1098/rsif.2011.0207)
- 42 Zar, J. H. 1999 Biostatistical analysis, pp. 732–738, 4th edn. London, UK: Prentice-Hall.
- 43 Sealy, S. G. 1996 Evolution of host defences against brood parasitism: implications of puncture-ejection by a small passerine. *Auk* **113**, 346–355.
- 44 Peer, B. D. & Sealy, S. G. 2000 Responses of scissor-tailed flycatchers (*Tyrannus forficatus*) to experimental cowbird parasitism. *Bird Behav.* **13**, 63–67.
- 45 Aviles, J. M., Soler, J. J., Soler, M. & Moller, A. P. 2004 Rejection of parasitic eggs in relation to egg appearance in magpies. *Anim. Behav.* **67**, 951–958. (doi:10.1016/j.anbehav.2003.08.022)
- 46 Antonov, A., Stokke, B. G., Moksnes, A. & Roskaft, E. 2008 Getting rid of the cuckoo *Cuculus canorus* egg: why do hosts delay rejection? *Behav. Ecol.* **19**, 100–107. (doi:10.1093/beheco/arm102)
- 47 Arias de Reyna, L. 1998 Coevolution of the great spotted cuckoo and its hosts. In *Parasitic birds and their hosts: studies in coevolution* (eds S. I. Rothstein & S. K. Robinson), pp. 129–142. Oxford, UK: Oxford University Press.
- 48 Ortega, C. P. & Ortega, C. P. 1998 *Cowbirds and other parasites*. Tuscon, AZ: University of Arizona Press.
- 49 Astie, A. A. & Reboreda, J. 2006 Costs of egg punctures and parasitism by shiny cowbirds (*Molothrus bonariensis*) at creamy-bellied thrush (*Turdus amaurochalinus*) nests. *Auk* **123**, 23–32. (doi:10.1642/0004-8038(2006)123[0023:COEPAP]2.0.CO;2)
- 50 Trivers, R. L. 1974 Parent–offspring conflict. *Am. Zool.* **14**, 249–264. (doi:10.1093/icb/14.1.249)
- 51 Hoover, J. P. 2003 Multiple effects of brood parasitism reduce the reproductive success of prothonotary warblers, *Protonotaria citrea*. *Anim. Behav.* **65**, 923–934. (doi:10.1006/anbe.2003.2155)
- 52 Hauber, M. E. 2003 Egg-capping is a cost paid by hosts of interspecific brood parasites. *Auk* **120**, 860–865. (doi:10.1642/0004-8038(2003)120[0860:EIACPB]2.0.CO;2)
- 53 Kilner, R. M. 2003 How selfish is a cowbird nestling? *Anim. Behav.* **66**, 569–576. (doi:10.1006/anbe.2003.2204)
- 54 Fraga, R. M. 1998 Interactions of the parasitic screaming and shiny cowbirds (*Molothrus rufoaxillaris* and *M. bonariensis*) with a shared host, the bay-winged cowbird (*M. badius*). In *Parasitic birds and their hosts: studies in coevolution* (eds S. I. Rothstein & S. K. Robinson), pp. 173–193. Oxford, UK: Oxford University Press.
- 55 Canestrari, D., Marcos, J. M. & Baglione, V. 2009 Cooperative breeding in carrion crows reduces the rate of brood parasitism by great spotted cuckoos. *Anim. Behav.* **77**, 1337–1344. (doi:10.1016/j.anbehav.2009.02.009)
- 56 Gloag, R., Tuero, D. T., Fiorini, V. D., Reboreda, J. C. & Kacelnik, A. 2011 The economics of nestmate-killing in avian brood parasites: a provisions trade-off. *Behav. Ecol.* (doi:10.1093/beheco/arr166)
- 57 Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man* (ed. B. Campbell), pp. 136–179. Chicago, IL: Aldine.
- 58 Smith, J. N. M. 1981 Cowbird parasitism, host fitness, and age of the host female in an island song sparrow population. *Condor* **83**, 152–161. (doi:10.2307/1367420)
- 59 Payne, R. B. & Payne, L. L. 1998 Brood parasitism by cowbirds: risks and effects on reproductive success and survival in indigo buntings. *Behav. Ecol.* **9**, 64–73. (doi:10.1093/beheco/9.1.64)
- 60 Sedgwick, J. A. & Iko, W. M. 1999 Costs of brown-headed cowbird parasitism to willow flycatchers. *Stud. Avian Biol.* **18**, 167–181.
- 61 Mason, P. & Rothstein, S. I. 1987 Crypsis versus mimicry and the color of shiny cowbird eggs. *Am. Nat.* **130**, 161–167. (doi:10.1086/284703)