



## Strategic egg destruction by brood-parasitic cowbirds?



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Obligate avian brood parasites do not provide direct care to their young but can indirectly increase their offspring's success in host nests. One way in which parasitic cowbirds (*Molothrus* sp.) could achieve this is through egg puncturing, whereby, prior to laying in a nest, females puncture the eggs that are already present in the nest to reduce the competition that their offspring will later face for food. In this study we investigated whether cowbirds strategically increase their puncturing effort with increasing competitiveness of the future brood. We filmed egg-puncturing behaviour by shiny cowbirds, *Molothrus bonariensis*, at nests of chalk-browed mockingbirds, *Mimus saturninus*, a large host whose nests often receive multiple cowbird eggs. We presented cowbirds with large (4 eggs) or small (1 egg) clutches of either mockingbird or cowbird eggs, where large clutch sizes predict greater intrabrood competition than small clutch sizes, and mockingbird eggs (which are larger) predict greater competition than other cowbird eggs. Cowbirds delivered more pecks and punctured more eggs per visit to larger clutches, and mockingbird eggs were broken more often than cowbird eggs, but pecked less per visit. The higher number of pecks aimed at cowbird eggs, despite these producing less competitive nestmates, could reflect responses to eggshell strength rather than egg size, as cowbird eggs are harder to break because of their thicker shells and so require more effort to puncture. Our results show that cowbird puncturing behaviour is not rigid and varies with nest contents. We suggest this variation is consistent with females increasing their offspring's chance of survival.

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Obligate avian brood parasites lay their eggs in nests of other species, hosts, which incubate the parasitic eggs and feed and protect the parasitic chicks until independence (Davies, 2000). Adult parasites therefore provide no direct care to their offspring, but they do have specialized behaviours that indirectly increase the chances that their young survive. Two such behaviours are the removal and the puncture of host eggs prior to the adult parasite laying its egg (Davies & Brooke, 1988; Dubina & Peer, 2013; Peer, 2006; Peer & Sealy, 1999; Spottiswoode & Colebrook-Robjent, 2007). This clutch reduction could benefit parasite young by increasing incubation efficiency, or by reducing the likelihood that hosts detect and reject the parasite egg (Davies, 2000; Peer & Bollinger, 2000), although for those parasitic species that are reared alongside other chicks in the nest the key function of clutch reduction behaviour is likely to be competition reduction. Parasites may benefit from having some nestmates when larger broods

increase parental care and when the parasites can outcompete the host's nestlings in the distribution of parental provisioning (Gloag, Tuero, Fiorini, Reboreda, & Kacelnik, 2012; Kilner, Madden, & Hauber, 2004), but when this is not the case, fewer nestmates increases parasite survival, because more of the food provisioned by host parents is available to parasite young (Carter, 1986; Fiorini, Tuero, & Reboreda, 2009; Gloag, Tuero, et al., 2012; Mason, 1986; Sealy, 1992; Soler et al., 2014).

The parasitic cowbirds (genus *Molothrus*, Icteridae) consist of five species (Lanyon, 1992; Price, Lanyon, & Omland, 2009) distributed across the Americas (Ortega, 1998). Cowbird nestlings are commonly reared alongside host young and other cowbird young (Ortega, 1998; but see Peer, Rivers, & Rothstein, 2013). Brown-headed cowbirds, *Molothrus ater*, remove host eggs, thus enhancing efficiency of incubation of the remaining clutch, including the parasite's egg (Peer & Bollinger, 2000). Egg removal also has consequences after hatching, modifying brood size and competition between nestlings, which can be favourable or unfavourable depending on the properties of the host–parasite system (Gloag, Tuero, et al., 2012). Shiny cowbirds, *Molothrus bonariensis*, are not known to remove eggs, but they render eggs unviable by puncturing. They, like bronzed cowbirds, *Molothrus*

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*aeneus*, thus modify the competition faced by their young from host chicks. In the case of hosts that are larger than the parasite, brood reduction increases the survivorship of the parasite nestlings (Carter, 1986; Fiorini et al., 2009; Gloag, Tuero, et al., 2012; Mermoz & Reboveda, 2003; Peer, 2006; Sackmann & Reboveda, 2003). The fitness implications of egg puncture are not straightforward, because the shiny cowbird is a generalist that uses host species both smaller and larger than themselves, and so its offspring share nests with host nestlings of differing competitive ability, as well as with other cowbirds. Gloag, Tuero, et al. (2012) modelled the impact of the presence of host nestlings on the rate of food intake for cowbird nestlings, taking both nestlings' competitive ability and host parents' responsiveness to nestlings into account. From the parasite's perspective there is an optimal number of host nestlings, depending on the interaction between a parasite's ability to stimulate parental provisioning and its ability to compete for food among the brood, and this optimum varies from host to host. Variation in the number of eggs in the host clutch at the time of parasitism has additional consequences for the survivorship of cowbird nestlings. Large clutches are more likely to have been completed and to have entered incubation, thus having a greater chance that the cowbird chick will hatch later and lose in competition with its nestmates (Carter, 1986; Fiorini et al., 2009). Egg destruction may also potentially increase the chance of nest desertion by the hosts (Rothstein, 1986), which could be an unintended consequence, but is unlikely to be the driving selective force in cases where the parasite lays her own egg after puncturing, as do shiny cowbirds, because desertion would then harm the attacking parasite's own direct fitness.

The variability in brood competitiveness experienced by cowbirds raises the opportunity for strategic flexibility in adult clutch reduction behaviour. That is, cowbirds could vary their puncturing behaviour according to the total number of eggs in a nest or the predicted relative competitiveness between host and parasite nestlings. The latter correlates with egg size, which is a strong predictor of hatchling size (Tuero, Fiorini, Mahler, & Reboveda, 2012). In the shiny cowbird, there is evidence to suggest that both egg number and egg size influence puncturing behaviour. Correlational data show that the number of eggs in a nest that are punctured prior to the appearance of the first parasitic egg increases with the number of host eggs in the nest (Fiorini et al., 2009; Tuero, Fiorini, & Reboveda, 2012). This is consistent with each cowbird puncturing more actively when clutches are larger, but could also arise because the larger the clutch, the more days have elapsed from the start of the host laying, and thus the more likely that multiple females have made puncture attacks (not all of which are followed immediately by laying). Correlational results have also shown that proportionally more host eggs are punctured per shiny cowbird egg laid in nests of a large host (chalk-browed mockingbirds, *Mimus saturninus*) than in nests of a small host (house wren, *Troglodytes aedon*; Fiorini et al., 2009; Tuero, Fiorini, Mahler, et al., 2012; Tuero, Fiorini, & Reboveda, 2012). Again, while this is consistent with flexibility in cowbird-puncturing behaviour, it could also be explained by differences between hosts in the number of females that visit the nest before the first event of parasitism occurs.

In this study we used direct observations of naturally occurring cowbird visits to host nests with experimentally manipulated clutches to investigate whether shiny cowbirds adjust their puncturing behaviour according to the size and/or number of eggs they encounter. We compared the number of total pecks that females made during each nest visit, using this variable as a measure of puncturing effort. We focused on within-host behavioural flexibility for cowbirds at nests of a single common host, the chalk-browed mockingbird. Nests of chalk-browed mockingbirds are

often multiply parasitized (Fiorini & Reboveda, 2006; Gloag, Fiorini, Reboveda, & Kacelnik, 2012). Eggs in the same nest are laid by different cowbird females, and females do not return to nests they have already parasitized to puncture eggs (Gloag, Fiorini, Reboveda, & Kacelnik, 2014). Thus, a female cowbird that arrives at a nest may encounter mockingbird eggs, other females' cowbird eggs, or a mixture of both. Mockingbird eggs are larger than cowbird eggs and represent a higher level of future food competition for the female's forthcoming offspring. Also, nestmates that hatch before cowbirds are significantly more competitive than those that hatch on the same day (Fiorini et al., 2009), where the likelihood of hatching asynchrony increases with increasing clutch size. If cowbirds respond to clutch properties, they may thus be expected to invest more puncturing effort in larger clutches and in clutches with a higher proportion of mockingbird eggs.

## METHODS

### *Study Site and Species Characteristics*

The study was carried out at Reserva El Destino, near the town of Magdalena, in Buenos Aires Province, Argentina (35°08'S, 57°23'W). The study site is a mosaic of grasslands and small patches of woodland, formed predominantly by talas (*Celtis ehrenbergiana*) and coronillos (*Scutia buxifolia*). The breeding season of both chalk-browed mockingbirds and shiny cowbirds at this site starts in mid-September and lasts until mid-January. The fieldwork was carried out during the reproductive seasons 2010–2011 and 2011–2012. We found mockingbird nests by focusing on individual activity and then inspecting potential nesting sites within the territory of breeding pairs. Mockingbirds build their nests in trees, constructing a large open cup of twigs lined with fibres and hair, where they lay three to five eggs (median = 4; Fiorini & Reboveda, 2006; Gloag, Fiorini, et al., 2012). Other quantitative parameters of the eggs, chicks and adults of cowbirds and mockingbirds are given in the Appendix.

### *Experimental Procedures and Filming*

We used nests during the mockingbird's laying period, when most cowbird parasitism occurs (Fiorini & Reboveda, 2006). We manipulated clutches at 131 nests to create four treatments, as shown in Table 1. The experimental clutches were small (1 egg) or large (4 eggs) and made of either mockingbird or cowbird eggs. Intra-brood competition is expected to increase with both clutch size and the proportion of mockingbird eggs in a clutch. Eggs used in experimental clutches were either laid naturally in the experimental nest, or transferred from other mockingbird nests at our study site. Most manipulations required either the addition or the removal of some eggs from the nest. When manipulations required egg removal, these eggs were maintained indoors at ambient temperature until the end of the experiment (from 1 to 4 days). Most of these eggs were then returned to their host nest of origin,

**Table 1**  
Experimental clutch compositions in nests of chalk-browed mockingbird used to assess shiny cowbird puncturing behaviour

| Egg species | Number of eggs          |                          |
|-------------|-------------------------|--------------------------|
|             | 1                       | 4                        |
| Mockingbird | Large egg, small clutch | Large eggs, large clutch |
| Cowbird     | Small egg, small clutch | Small eggs, large clutch |

Nest contents were manipulated to create small clutches (1 egg) or large clutches (4 eggs) of large eggs (mockingbird) or small eggs (cowbird).

the exception being some cowbird eggs that were retained for other experiments. Eggs that were returned to the nest hatched successfully and in time for the chicks to be successfully reared.

We recorded cowbird visits to mockingbird nests using micro-cameras with infrared lights (handycam high-resolution CCD colour) placed approximately 20 cm above each nest. Each micro-camera was connected to a video recorder (Lawmate PVR-1000 or PVR500 ECO) and to a battery hidden under the grass and branches on the ground. Female cowbird visits can be categorized into two classes (Gloag, Fiorini, Reboresda, & Kacelnik, 2013): (1) laying visits, which occur prior to sunrise, in which cowbirds first puncture eggs and then lay their own egg and (2) puncturing visits, which occur throughout the rest of the day, in which cowbirds puncture eggs but do not lay an egg. A cowbird female may make one or two puncturing visits to a mockingbird nest prior to egg laying, but she does not return to nests for further puncturing or laying once she has laid an egg (Gloag et al., 2014). Nests were filmed from 1 h prior to sunrise until either mid-morning, when we performed the first nest check, or sunset, when we performed a second nest check, on each day. We filmed nests until the end of the first day of incubation, or until the nest was predated or abandoned. During nest checks we marked new eggs, recorded egg punctures and egg losses, added or removed eggs as necessary, and serviced the equipment.

For analysis we considered only the first cowbird visit per recording session (a session spanned the time between two nest checks). This was because the first cowbird to arrive at the nest could puncture an egg or lay her own egg, thus changing the composition of the experimental clutch encountered by those arriving later. Of 131 experimental nests, we filmed one or more cowbird visits at 110 (84%). Of 162 total cowbird visits filmed, we used 135 for analysis, with the remaining 27 visits excluded because the presence of mockingbirds prevented a clear view of the cowbird's beak and hence the quantification of pecking behaviour. We estimate that there were between 35 and 49 different female cowbirds using mockingbird nests at our study site (Gloag et al., 2014), so each female was likely to have been filmed multiple times. A subset of these females were individually marked (11 females, making 40 visits), but most could not be individually identified (95 visits). The use of visits rather than female numbers as the unit of analysis may have caused some pseudoreplication, but this was unavoidable and, given our large samples, was unlikely to be responsible for the effects reported later.

We counted the total number of pecks that a cowbird made and the number of pecks received by each individual egg per visit. We defined one peck as the movement of a female cowbird's head that brought her beak into contact with an egg. When necessary, videos were viewed in slow motion to count pecks. Some, but not all, pecks result in an egg being punctured (i.e. broken), but this is not always detectable until the clutches are physically examined. For the analysis of egg punctures, we excluded recording sessions with multiple visits by cowbirds, because in those cases we could not discern which visit was responsible for the egg puncture.

All work complied with the Argentinean Law for the Conservation of Wild Fauna (Ley Nacional de Fauna 22421/81), and was undertaken under license from the Organismo Provincial de Desarrollo Sostenible, Argentina (Permit number 202/12-O.P.D.S.).

### Statistical Analyses

Cowbird females arriving at mockingbird nests are often mobbed by one or both hosts. This does not reduce the frequency of their egg laying or puncture attacks, but it does decrease the probability of breaking at least one egg (Gloag et al., 2013). Therefore, we included mobbing as a predictor variable in our

models, classifying each cowbird visit as either mobbed or not mobbed.

Our videos captured cowbirds puncturing eggs, both during 'laying' and 'puncturing' visits (Gloag et al., 2013). In our data set, cowbirds were frequently mobbed during laying visits (51/82 = 64%) but seldom during puncturing visits (7/53 = 13%). For this reason, type of visit and occurrence of mobbing were highly correlated (chi-square test:  $\chi^2_1 = 31.5$ ,  $P < 0.00001$ ). To avoid the effect of this correlation on model fitting (Logan, 2010), we included mobbing but not visit type as a predictor. That is, we assumed that cowbird puncturing behaviour is similar regardless of whether or not it is followed by egg laying. In support of this assumption, when we considered only nests in which no mobbing occurred, we found that visit type did not affect the number of punctured eggs (GLM: intercept: estimate =  $-0.50$ ,  $P = 0.003$ ; type of visit: estimate =  $-0.18$ ,  $P = 0.8$ ,  $N = 56$  visits).

We also assumed that all females that were filmed making puncturing visits intended to return to the same nest to lay. Gloag et al. (2014) found that 65% of puncturing visits were followed by laying visits by the same female. Where females do not return to lay after a puncturing visit, it may be simply because they chose to lay instead in a different nest. It is also possible however that some puncturing visits were made by females that were not ready to lay and that they punctured eggs as an adaptation to induce hosts to re-nest, thus creating an opportunity for later laying (Arcese, Smith, & Hatch, 1996; Nakamura & Cruz, 2000), or that they served to assess the incubation status of eggs and thus the readiness of the nest for parasitism (Massoni & Reboresda, 1999). We return to these possibilities later.

We used generalized linear models (Crawley, 2007; Logan, 2010; McCullagh & Nelder, 1989) to test the effect of clutch size, clutch type and mobbing on the following dependent variables: (1) total number of pecks (GLM with negative binomial error distribution and log link function), (2) mean number of pecks per egg (GLM with Gaussian error distribution and identity link function), (3) probability of breaking at least one egg per cowbird visit (GLM with binomial error distribution and logit link function) and (4) number of eggs broken (GLM with Poisson error distribution and log link function). We estimated overdispersion for the models with negative binomial and Poisson distributions, and in all cases the parameter was close to 1, indicating that the models fitted their distribution. The predictors clutch size, clutch type and mobbing were included as categorical factors, and all pairwise interaction terms were evaluated as additional predictor variables. To evaluate statistical significance we used the hypothesis test, and to simplify models we used the backward stepwise method. When an interaction term was significant, we analysed the effect of our principal variables in each level of the other interaction term. We used the sequential Bonferroni test to adjust for the number of simultaneous tests (Rice, 1989).

Statistical analyses were carried out using R software, version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria). Values are reported as means  $\pm$  SE. All tests were two tailed, and differences were considered significant at  $P < 0.05$ .

## RESULTS

### Pecking Behaviour of Shiny Cowbirds

#### Descriptive variables

All filmed cowbird visits were of female cowbirds, with an average visit duration of  $23.5 \pm 2.0$  s (range 1–177 s,  $N = 135$ ). The cowbird's beak made contact with eggs in 80% of visits (108/135). The latency to the first peck was  $2.1 \pm 0.3$  s (range 0–23 s,  $N = 108$ ), and a puncture attack (interval between the first and the last peck)

lasted on average  $11.6 \pm 1.63$  s (range 1–104 s,  $N = 108$ ). These values depended however on whether cowbirds were mobbed or not (latency to peck: not-mobbed visits:  $2.6 \pm 0.4$  s,  $N = 73$ ; mobbed visits:  $1.1 \pm 0.3$  s,  $N = 35$ ;  $F_{1,106} = 6.5$ ,  $P = 0.01$ ; puncture attack duration: not-mobbed visits:  $15.9 \pm 2.3$  s,  $N = 73$ ; mobbed visits:  $2.6 \pm 0.6$  s,  $N = 35$ ;  $F_{1,106} = 16.7$ ,  $P < 0.001$ ). On mobbed visits, the mean time between the first peck and the arrival of mockingbirds was  $3.8 \pm 0.7$  s ( $N = 35$ ). The cowbirds pecked eggs on average at a rate of  $2.3 \pm 0.2$  pecks/s ( $N = 108$ ; Supplementary Material Video S1), but the rate of pecks varied considerably and was sometimes very high (range 0.4–11 pecks/s; Supplementary Material Video S2). When cowbirds were mobbed, they frequently stopped pecking just before the mockingbirds entered the filming field, but occasionally they continued pecking with mockingbirds delivering beak blows to their head and body (time between last peck and start of mobbing =  $1.2 \pm 0.6$ , range –6 to 10 s,  $N = 35$ ). On laying visits, the cowbirds stayed at the nest for an average of  $17.1 \pm 3.3$  s after a puncture attack had ceased (range 0–175 s,  $N = 57$ ) during which time they laid their egg and/or continued to be mobbed by the mockingbird until departure.

#### Total number of pecks per cowbird visit

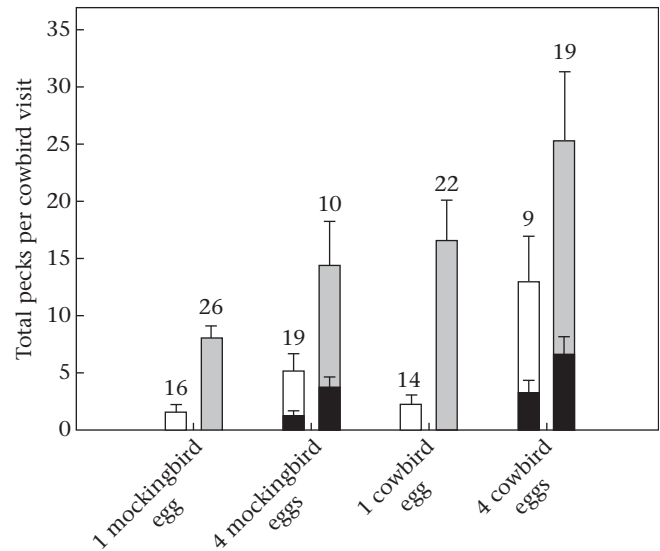
Clutch type and the interaction between clutch size and mobbing were significant predictors of the total number of pecks that a cowbird directed at eggs during a nest visit (Table 2). Cowbird eggs were pecked more than mockingbird eggs (Fig. 1). To understand the effect of the interaction term clutch size\*mobbing, we first considered only nests where female cowbirds were not mobbed. In these cases, the number of pecks was higher in four-egg clutches than in one-egg clutches and in cowbird clutches than in mockingbird clutches (GLM: intercept: estimate = 2.12,  $P < 0.0001$ ; clutch size (4 eggs): estimate = 0.49,  $P = 0.03$ ; clutch type (cowbird eggs): estimate = 0.66,  $P = 0.003$ ; Fig. 1). These trends were similar when we considered only nests where female cowbirds were mobbed, although the reliability of the clutch type effect dropped below conventional significance (GLM: intercept: estimate = 0.31,  $P = 0.35$ ; clutch size (4 eggs): estimate = 1.44,  $P = 0.0001$ ; clutch type (cowbird eggs): estimate = 0.65,  $P = 0.09$ ; Fig. 1). When we considered only nests containing one egg, the number of pecks was higher in not-mobbed visits than in mobbed visits and in cowbird clutches than in mockingbird clutches (GLM: intercept: estimate = 2.14,  $P < 0.0001$ ; mobbing (presence): estimate = –1.82,  $P < 0.0001$ ; clutch type (cowbird eggs): estimate = 0.61,  $P = 0.02$ ;

**Table 2**

Estimates  $\pm$  SE, Z values and significance ( $P$ ) of predictor variables included in the final models for total number of pecks, mean number of pecks per egg, probability of breaking (i.e. puncturing) at least one egg and number of broken eggs by female shiny cowbirds per visit to chalk-browed mockingbird nests

| Response variable                        |                              | Estimate $\pm$ SE | Z     | P        |
|--|------------------------------|-------------------|-------|----------|
| Total number of pecks                    | Intercept                    | 2.11 $\pm$ 0.17   | 12.29 | <0.0001* |
|  | Clutch type (cowbird eggs)   | 0.66 $\pm$ 0.19   | 3.49  | 0.0006*  |
|  | Clutch size $\times$ mobbing | 0.96 $\pm$ 0.39   | 2.51  | 0.01*    |
| Mean number of pecks per egg             | Intercept                    | 10.03 $\pm$ 1.30  | 7.71  | <0.0001* |
|  | Clutch type (cowbird eggs)   | 4.25 $\pm$ 1.39   | 3.96  | 0.003*   |
|  | Clutch size $\times$ mobbing | 8.10 $\pm$ 2.81   | 2.88  | 0.005*   |
| Probability of breaking at least one egg | Intercept                    | 1.66 $\pm$ 0.63   | 2.64  | 0.008*   |
|  | Clutch size (4 eggs)         | 1.83 $\pm$ 0.54   | 3.39  | 0.0007*  |
|  | Mobbing $\times$ clutch type | 2.28 $\pm$ 1.11   | 2.05  | 0.04*    |
| Number of broken eggs                    | Intercept                    | –0.30 $\pm$ 0.24  | –1.24 | 0.22     |
|  | Clutch type (cowbird eggs)   | –1.47 $\pm$ 0.33  | –4.42 | <0.0001* |
|  | Clutch size (4 eggs)         | 1.08 $\pm$ 0.29   | 3.70  | 0.0001*  |
|  | Mobbing (presence)           | –1.05 $\pm$ 0.30  | –3.45 | 0.0004*  |

Asterisks denote significance after Bonferroni correction.



**Figure 1.** Number of total pecks directed at treatment clutches by shiny cowbirds per visit to chalk-browed mockingbird nests. White bars indicate that cowbirds were not mobbed by mockingbirds at the nest and grey bars indicate that they were not mobbed. For clutches of four eggs, black bars indicate the mean number of pecks received on average by each egg of the clutch. Means  $\pm$  SE are represented. Sample sizes are given above the bars.

Fig. 1), and this was also true when considering only nests with four eggs (GLM: intercept: estimate = 2.56,  $P < 0.0001$ ; mobbing (presence): estimate = –0.85,  $P = 0.002$ ; clutch type (cowbird eggs): estimate = 0.73,  $P = 0.007$ ; Fig. 1).

#### Mean number of pecks per egg

Mean number of pecks per egg was affected by clutch type and by the interaction between clutch size and mobbing (Table 2). In the overall analysis, cowbird eggs were pecked more than mockingbird eggs (Fig. 1). To understand the interaction term clutch size\*mobbing, we first analysed only nests where cowbirds were not mobbed. In these cases, the mean number of pecks was higher in cowbird clutches than in mockingbird clutches and in one-egg clutches than in four-egg clutches (GLM: intercept: estimate = 9.02,  $P < 0.0001$ ; clutch type (cowbird eggs): estimate = 6.46,  $P = 0.007$ ; clutch size (4 eggs): estimate = –7.86,  $P = 0.001$ ; Fig. 1). However, in nests where cowbirds were mobbed, the mean number of pecks per egg was not affected by clutch size and the reliability of the clutch type effect dropped below conventional significance (GLM: intercept: estimate = 1.29,  $P = 0.03$ ; clutch size (4 eggs): estimate = 0.23,  $P = 0.73$ ; clutch type (cowbird eggs): estimate = 1.24,  $P = 0.08$ ; Fig. 1). In one-egg clutches, the mean number of pecks was lower in mobbed visits than in not-mobbed visits and in mockingbird clutches than in cowbird clutches (GLM: intercept: estimate = 9.47,  $P < 0.0001$ ; mobbing (presence): estimate = –10.16,  $P < 0.0001$ ; clutch type (cowbird eggs): estimate = 5.49,  $P = 0.01$ ; Fig. 1). The same pattern was found in four-egg clutches (GLM: intercept: estimate = 384,  $P = 0.001$ ; mobbing (presence): estimate = –2.68,  $P = 0.03$ ; clutch type (cowbird eggs): estimate = 2.36,  $P < 0.05$ ; Fig. 1).

#### Puncture Success of Shiny Cowbirds

##### Probability of breaking at least one egg per cowbird visit

Clutch size and the interaction between mobbing and clutch type were significant predictors of the probability of breaking at least one egg per cowbird visit (Table 2). Four-egg clutches had a higher probability of having at least one egg broken than one-egg clutches

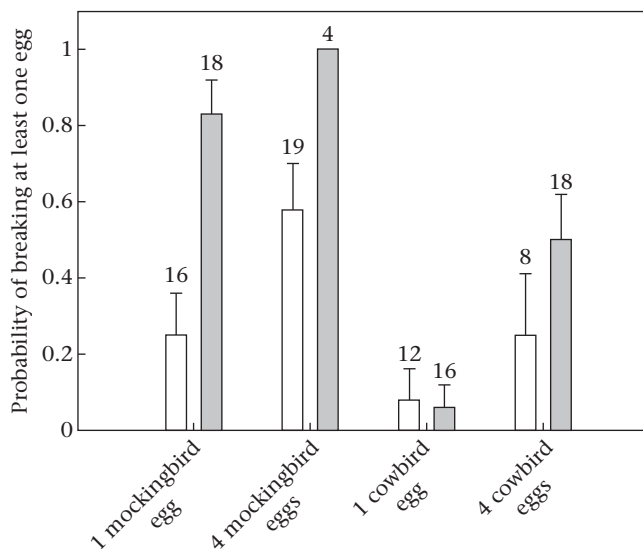
(Fig. 2). To understand the effect of the interaction term mobbing\*clutch type, we first considered in the analyses only nests where cowbirds were not mobbed. In these cases, the probability of breaking at least one egg was higher in mockingbird clutches than in cowbird clutches and in four-egg clutches than in one-egg clutches (GLM for not mobbed nests: intercept: estimate = 1.63,  $P = 0.009$ ; clutch type (cowbird eggs): estimate =  $-4.39$ ,  $P = 0.0002$ ; clutch size (4 eggs): estimate = 2.77,  $P = 0.01$ ; Fig. 2). These trends were similar when we considered only nests where female cowbirds were mobbed (GLM for mobbed nests: intercept: estimate =  $-1.08$ ,  $P = 0.04$ ; clutch type (cowbird eggs): estimate =  $-1.37$ ,  $P < 0.05$ ; clutch size (4 eggs): estimate = 1.39,  $P = 0.03$ ; Fig. 2). When we considered only those nests that contained cowbird eggs, the probability of breaking at least one egg was higher in four-egg clutches than in one-egg clutches, but it was not affected by the presence of mockingbird mobbing (GLM: intercept: estimate =  $-2.31$ ,  $P = 0.003$ ; clutch size (4 eggs): estimate = 2.21,  $P = 0.009$ ; mobbing (presence): estimate =  $-0.7$ ,  $P = 0.36$ ; Fig. 2), while in nests that contained mockingbird eggs, the probability of breaking at least one egg was higher in four-egg clutches than in one-egg clutches and in not-mobbed visits than in mobbed visits (GLM: intercept: estimate = 1.65,  $P = 0.007$ ; clutch size (4 eggs): estimate = 1.51,  $P = 0.03$ , mobbing (presence): estimate =  $-2.83$ ,  $P = 0.0006$ ; Fig. 2).

#### Number of broken eggs per cowbird visit

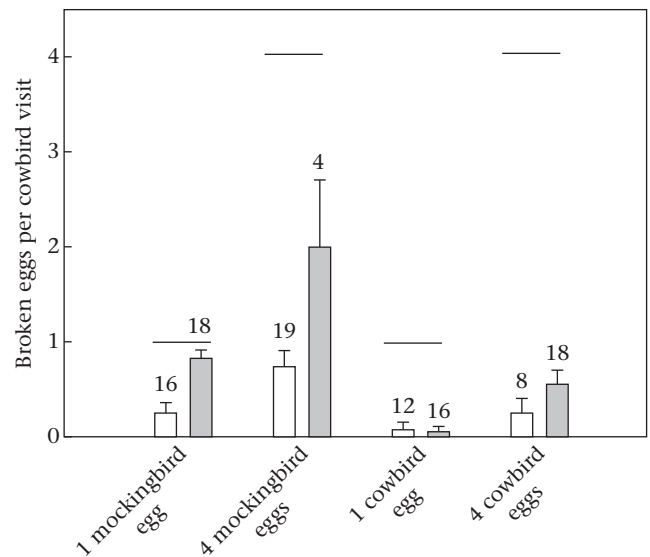
In the final model, clutch type, clutch size and mobbing remained as significant predictors of the number of successfully punctured (e.g. broken) eggs per female cowbird visit (Table 2). Cowbirds broke more eggs in four-egg clutches when the clutch comprised mockingbird eggs and when mockingbirds did not mob the cowbird at the nest (Fig. 3).

## DISCUSSION

By varying the number and type of eggs in natural nests of a common host, the chalk-browed mockingbird, we show that the puncturing behaviour of female shiny cowbirds is not indiscriminate, but rather varies according to the contents of the nest.



**Figure 2.** Probability that at least one egg of the clutch was broken (e.g. punctured) during a female shiny cowbird visit to a chalk-browed mockingbird nest. White bars indicate that cowbirds were mobbed by mockingbirds at the nest and grey bars indicate that they were not mobbed. Means  $\pm$  SE are represented. Sample sizes are given above the bars.



**Figure 3.** Number of eggs broken (e.g. punctured) by shiny cowbirds per visit to chalk-browed mockingbird nests, where cowbirds were mobbed (white bars) or not mobbed (grey bars) by mockingbirds at the nest. Horizontal lines above the bars indicate the maximum number of eggs that cowbirds could break when visiting the nest. Means  $\pm$  SE are represented. Sample sizes are given above the bars.

Cowbirds delivered more pecks to clutches with four eggs than they did to those with a single egg. They also delivered more pecks when clutches comprised only cowbird eggs than when clutches comprised only mockingbird eggs. Below we discuss whether the variation in puncturing behaviour we observed represents behavioural flexibility by cowbirds and what may be its adaptive significance.

#### Variation in Puncturing Behaviour with Clutch Size

Shiny cowbirds delivered more pecks per visit to four-egg clutches than they did to one-egg clutches, and more eggs were broken in four-egg clutches. Cowbirds delivered fewer mean pecks per egg in larger clutches, but their probability of breaking at least one egg during a nest visit was higher in four-egg clutches than in one-egg clutches.

We propose that a greater puncturing effort in large clutches is adaptive for cowbird females mainly because the larger a brood, the more competition each nestling faces for food. Since host parent provisioning is likely to increase less than proportionally with brood size (slope  $< 1$ ), the marginal cost imposed by each additional nestling should increase with clutch size, thus increasing the benefit of destroying one egg in a four-egg clutch with respect to destroying one egg in a one-egg clutch. This assumes however that the clutch sizes encountered at the time of parasitism will not change, whereas a nest with one egg most likely signifies that remaining eggs have yet to be laid. More critically therefore for cowbird females facing large clutches is not the number of eggs per se, but that they signify a later stage in host laying and thus a greater chance of hatching asynchrony for their offspring. Given that mockingbird young are large, a failure to puncture eggs in this host when laying asynchronously almost always results in cowbird nestling death (Fiorini et al., 2009). It has also been argued that the risk of nest abandonment by the hosts may be higher following egg loss in small clutches than in large clutches, and this may act additionally and independently of nestling competition in determining the optimal clutch reduction for a given clutch size, since parasites that cause abandonment lose the investment on their

own egg (Davies & Brooke, 1988; Guigueno & Sealy, 2011; Massoni & Reboreda, 1998; Nakamura & Cruz, 2000; Peer & Sealy, 1999; Sealy, 1992; Wiley, 1988). In great spotted cuckoos, *Clamator glandarius*, the number of eggs destroyed in nests of their host, the magpie, *Pica pica*, increased with magpie clutch size, and this increased the parasite's reproductive success (Soler, Soler, & Martínez, 1997). Spottiswoode and Colebrook-Robjent (2007) also reported a similar trend for the greater honeyguide, *Indicator indicator*, which punctured more eggs when laying occurred late relative to that of the host.

Does variation in peck number with clutch size indicate behavioural flexibility on the part of cowbirds? A difference in pecking effort and success could also arise if cowbirds followed a simple rule of 'peck each egg until it breaks'. In this case, cowbirds would stop pecking after breaking an egg in a one-egg clutch, but would proceed to peck the next egg in a four-egg clutch. There is little evidence however that cowbirds cease pecking at eggs once punctured, given the frantic nature of puncture attacks (particularly where host mobbing occurs), and that broken eggs regularly receive multiple punctures. Also in captivity, female cowbirds continue pecking at eggs they have already punctured (Cossa, 2013). On the other hand, cowbirds frequently do not puncture all of the eggs in the clutch (see Fig. 3), indicating that they do not need to see that all the eggs of the clutch are broken in order to stop pecking. It seems most likely, therefore, that the difference in pecking we detected between clutch sizes reflects a flexible response of cowbirds to clutch size.

Cowbirds could also use egg puncture behaviour to assess the degree of development of the host's embryos (egg-testing hypothesis; Massoni & Reboreda, 1999) or, if nests are discovered when they are too advanced to parasitize, to force re-nesting and thus provide new opportunities for parasitism (nest predation hypothesis; Arcese et al., 1996; Nakamura & Cruz, 2000). However, the egg-testing hypothesis does not predict differences in puncture behaviour depending on clutch size, and the nest predation hypothesis seems not very relevant to our system because in mockingbirds most puncture attacks occur during the laying period (Fiorini & Reboreda, 2006) and are followed by egg laying, either immediately or in subsequent days (Gloag et al., 2014).

#### Variation in Puncturing Behaviour with Egg Size

Since larger eggs produce larger nestlings and hence tougher competition, cowbirds could be expected to peck more when confronted with mockingbird eggs than when confronted with cowbird eggs, but we found the opposite to be the case. It may be argued that competition other than that for provisioned food drives pecking behaviour, increasing the benefit of eliminating future adult cowbirds while allowing the survival of future hosts, but this is vulnerable to the usual weaknesses of group selection reasoning (the benefit of affecting future numbers of hosts and parasites will be shared by the cowbird population while the behaving female pays the cost of puncturing). Notably, mockingbird eggs were about four times more likely to be punctured than cowbird eggs, despite the higher number of pecks delivered to eggs in cowbird clutches. In this comparison, therefore, pecking effort was not correlated with puncture success. An alternative, mechanistic, explanation then may be that cowbird eggs attract a greater puncturing effort because they have thicker shells (Mermoz & Ornelas, 2004; Picman, 1989; Spaw & Rohwer, 1987) and are more resistant to puncture than mockingbird eggs (López, 2013). Thus, while our results reveal that egg characteristics of the clutch can affect puncturing behaviour of female cowbirds parasitizing this host, the extent to which egg size specifically determines puncturing behaviour remains open.

Studies in a number of parasite–host systems have reported that nest parasites remove or puncture more eggs when targeting larger hosts than when targeting smaller hosts (Blankespoor, Oolman, & Uthe, 1982; Røskft, Orians, & Beletesky, 1990; Sealy, 1992; shiny cowbirds: Fiorini et al., 2009). Assuming individual females target hosts of different sizes (Alderson, Gibbs, & Sealy, 1999; Mahler, Confalonieri, Lovette, & Reboreda, 2007; Woolfenden, Gibbs, Sealy, & McMaster, 2003), cowbirds might use egg size to gauge how forcefully to puncture when visiting different hosts, but other host-specific cues, such as nest type or host size, could equally serve to produce variation in pecking behaviour between hosts.

A higher peck number directed at other cowbird eggs would help to drive selection for increased eggshell strength in cowbirds (Brooker & Booker, 1991; Hudson, 1874; Mermoz & Ornelas, 2004). In other parasites, the increased strength of parasitic eggs has been largely attributed to the pressure of host defences, whereby stronger eggs better evade puncture-rejection by hosts. In shiny cowbirds, and probably other cowbirds, where multiple parasitism is common, intraspecific competition is probably paramount in shaping eggshell strength (Brooker & Booker, 1991; Mermoz & Ornelas, 2004). Similarly, Spottiswoode (2013) recently proposed that the selection pressure for egg characteristics of the parasitic greater honeyguides has arisen by interference competition among parasites themselves.

#### Final Remarks

In this study, we controlled for mobbing by mockingbirds when assessing puncturing behaviour, because mobbing significantly reduces the effectiveness of cowbird puncture attacks (Gloag et al., 2013). It is possible that other variables, not evaluated in this study can also influence shiny cowbird puncture behaviour or puncture success in mockingbird nests. For example, clutch characteristics and the presence of host mobbing could affect the force that the parasitic female uses to peck the eggs. In our study, although the mean number of pecks delivered to cowbird eggs in one-egg clutches in the absence of mobbing was similar to the number of pecks delivered to four cowbird eggs in four-egg clutches during mobbed visits, the average number of broken cowbird eggs in one-egg clutches was four times lower than that of four-egg clutches. This result suggests that, under some circumstances, the motivation of the female cowbird can be manifest in variables other than the number of pecks she makes (e.g. the force at which she delivers each blow). These aspects of puncturing flexibility remain for future study.

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#### Supplementary Material

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## Appendix

Table A1

Quantitative parameters of eggs, chicks and adults of the brood-parasitic shiny cowbird and a common host, the chalk-browed mockingbird

| Variable                                     | Shiny cowbird            | Chalk-browed mockingbird |
|--|--------------------------|--------------------------|
| Egg volume (cm <sup>3</sup> ) <sup>a,b</sup> | 4.5±0.1                  | 5.7±0.1                  |
| Eggshell thickness (µm) <sup>b</sup>         | 118±7                    | 94±19                    |
| Egg strength (N) <sup>b</sup>                | 3.62±0.07*               | 2.20±0.02*               |
| Chick weight at hatching (g) <sup>c,d</sup>  | 4.9±0.2                  | 6.1±0.1                  |
| Adult weight (g) <sup>e,f</sup>              | ♀: 46.8±1.4; ♂: 51.6±1.4 | 78.7±4.7                 |
| Sexual dichromatism <sup>e,f</sup>           | Present                  | Absent                   |

<sup>a</sup> Tuero, Fiorini, Mahler, et al. (2012); <sup>b</sup> López (2013); <sup>c</sup> Tuero et al. (2013); <sup>d</sup> Fiorini (2007); <sup>e</sup> Reboreda et al. (1996); <sup>f</sup> Fraga (1985).

\* Estimations performed through quasistatic punctures (López, 2013).