



## Resistance is futile: prohibitive costs of egg ejection in an obligate avian brood parasite host

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Most hosts of the parasitic brown-headed cowbird, *Molothrus ater*, demonstrate an astonishing lack of defences against parasitism, typically explained by evolutionary lag. We investigated antiparasite strategies of the dickcissel, *Spiza americana*, whose apparent acceptance of parasitism is unlikely to be explained by lag because its historic centre of abundance overlaps with that of the cowbird. Cowbirds parasitized almost half of dickcissel nests (343 of 767 nests), and dickcissels suffered significant costs when attempting to eject cowbird eggs. Our predicted responses indicated that acceptance of parasitism would lead to the loss of 0 hosts eggs, attempted ejections would lead to the loss of 1.2 host eggs and successful ejection of cowbird eggs would lead to the loss of 1.6 host eggs. There was no significant cost of raising a single cowbird nestling, but parasitized nests had 1.1 fewer host eggs due to removal by female cowbirds or when the thick-shelled cowbirds eggs struck the host eggs during laying. After accounting for damaged eggs that still hatched, acceptance of parasitism yielded a loss of 1.1 eggs/nestlings, those that attempted to eject the cowbird egg lost 1.8 eggs/nestlings and those that ejected the cowbird egg lost 2.0 eggs/nestlings. The prohibitive costs of egg ejection combined with the relatively low costs of raising a cowbird nestling may explain why most dickcissels (64%) accepted parasitism or stopped trying to eject cowbird eggs. However, some birds persisted in their ejection attempts, so there are likely additional carryover fitness effects on hosts of raising and sharing nests with cowbirds. Because of the difficulty in ejecting cowbird eggs, dickcissels would benefit from a strategy that emphasizes frontline defences to prevent parasitism from occurring in the first place.

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Animals are expected to behave optimally to maximize their fitness as a result of natural selection (Dall, Giraldeau, Olsson, McNamara, & Stephens, 2005; Houston, McNamara, & Steer, 2007; McNamara & Houston, 2009). Obligate avian brood parasitism reduces host fitness (Davies, 2000), and hosts should evolve defences to lessen these costs, although there may be some instances where acceptance can be induced (Chakra, Hilbe, & Traulsen, 2016; Hoover & Robinson, 2007). Many common cuckoo, *Cuculus canorus*, hosts eject nonmimetic eggs and some also adjust their response to parasitism based on the costs of ejection (Antonov, Stokke, Moksnes, & Røskaft, 2009; Davies, Brooke, & Kacelnik, 1996). In contrast, hosts of the brown-headed cowbird, *Molothrus ater* (hereafter 'cowbird'), show an astonishing lack of defensive behaviour towards parasitism and almost

no evidence of flexibility in their response to it (Peer & Sealy, 2004a; Rothstein, 1975). Unlike cuckoos, cowbirds do not evict host nestlings and the host nestlings must compete with the cowbird nestlings, although in many instances only the cowbird survives (Peer, Rivers, Merrill, Robinson, & Rothstein, 2018; Peer, Rivers & Rothstein, 2013). Cowbirds and their hosts have also coevolved for less than half the time as cuckoos and their hosts (2.8–3.8 Ma and 6.3–8.4 Ma, respectively; Rothstein, Patten, & Fleischer, 2002). As a result, this apparent maladaptive behaviour by cowbird hosts has largely been assumed to be a consequence of evolutionary lag (Peer & Sealy, 2004a; Rothstein, 1975a), whereby hosts have not yet evolved defences, and once the appropriate mutation occurs, ejection of parasite eggs will spread rapidly throughout host populations (Rothstein, 1975b).

Evolutionary lag can account for acceptance of parasitism by hosts that nest in heavily forested habitats rarely penetrated by cowbirds (Hosoi & Rothstein, 2000; Peer & Sealy, 2004a). However, hosts that have nested within the historic centre of cowbird

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abundance have been exposed to parasitism for a much longer time and should be the species most likely to express sophisticated responses to parasitism (Hosoi & Rothstein, 2000; Mayfield, 1965; Peer, Robinson, & Herkert, 2000). Therefore, lag is not likely the best explanation to account for their acceptance. Instead, it is possible that in some cases acceptance of parasitism may be the superior strategy depending on the costs of expressing defences relative to their benefits. Costs associated with egg ejection include recognition and rejection errors. Recognition errors occur when hosts eject their own eggs from a parasitized nest and are more common when the parasite lays an egg that resembles the host's eggs (Davies & Brooke, 1988; Davies et al., 1996). Rejection errors take place when a host damages its own eggs in the process of removing the parasitic egg and are more likely to occur in hosts with small bills that puncture-eject parasite eggs (Rohwer & Spaw, 1988; Rothstein, 1975a; Røskaft, Rohwer, & Spaw, 1993). Parasites have thick-shelled eggs (Antonov et al., 2013; Jaeckle et al., 2012; Picman, 1989), and bills of small-billed hosts bounce off the parasite egg and onto their own eggs, causing damage to them (Antonov et al., 2013; Antonov et al., 2009; but see Sealy, 1996). If the costs of raising a brood parasite are relatively small, but the costs associated with egg ejection are excessive, then a host may maximize its fitness by tolerating parasitism (Rohwer & Spaw, 1988). Here we demonstrate, for the first time, the prohibitive costs of egg ejection in a cowbird host, the dickcissel, *Spiza americana*, which combined with the relatively low costs of raising a cowbird nestling may favour acceptance of parasitism in some instances.

## METHODS

### Experimental Procedures

Dickcissel nests were monitored for cowbird parasitism and were experimentally parasitized in McDonough County, Illinois, U.S.A. (40°16'48"N, 90°43'48"W) during 2006–2008. Nests were parasitized with real cowbird eggs collected from other nests freshly laid, or with artificial eggs composed of plaster of Paris that were similar to real cowbird eggs (Peer, Kuehn, Rothstein, & Fleischer, 2011; Rothstein, 1975a). A subsample ( $N = 10$ ) of our artificial cowbird eggs measured  $21.3 \times 16.4$  mm and weighed 3.6 g. Real cowbird eggs measure  $21.5 \times 16.4$  mm and weigh 3.0 g (Lowther, 1993). Dickcissels lay blue eggs, whereas cowbird eggs have a white background with brown and grey spots. No host eggs were removed in conjunction with experimental parasitism because this behaviour varies and there is little evidence it affects cowbird host response (Rothstein, 1975a). Eggs were added during laying (53%) and early incubation (47%) when cowbirds typically lay (Rothstein, 1975a). Nests were checked for host response and natural parasitism every 1–3 days, although most (>90%) were checked daily. Eggs in control nests were monitored for damage and treated in the same manner as experimental nests except no parasitic eggs were added to them. We did not control for the possibility that flushing hosts during experimental parasitism could influence ejection behaviour, which has been shown to occur in a single cuckoo host (Hanley et al., 2015). Eggs were considered 'ejected' if they were removed from the nest and they were considered 'accepted' if they remained in the nest for at least 5 days with parents still attending the nest and no host eggs disappeared or were damaged following parasitism (Rothstein, 1975a). Responses were considered 'attempted ejections' when cowbird eggs were not removed from the nest. In these cases, artificial cowbird eggs had peck marks or paint chipped off by the host's bill, or host eggs were damaged after the host's bill bounced off the real or artificial cowbird eggs onto their own eggs, causing them to break or dent (see below). The number of host eggs lost per ejection attempt included those that

were broken, dented or found below the nest. We interpreted these as losses because eggs were never found below unmanipulated control nests, nor were damaged eggs found in control nests (see below). Any nebulous results were excluded from analysis.

### Nestling Losses

Collecting nestling data at our primary study site was not logistically feasible, thus we examined nestling mortality (number of nestlings failing to fledge) in 466 dickcissel nests monitored in 2001 and 2002 across eight sites in eastern Kansas and northeastern Oklahoma (Jensen & Cully, 2005). No experimental manipulations of nests were conducted and nest contents were monitored every 3–4 days until nest completion. Nests used for analysis were active during the nestling stage and where incubation or hatch day were observed so that the number of host and cowbird offspring entering the nestling stage was known. If incomplete hatch was observed prior to finding the nest empty on the subsequent visit (i.e. depredation), these nests were omitted from analyses because the number of hatched host and cowbird young entering the nestling stage was uncertain. Nestling histories therefore accounted for losses of dickcissel young due to starvation and removal by parents as well as partial and complete depredation by predators.

### Statistical Analyses

We predicted that the cost of parasitism (number of host eggs lost) would be driven by host response (accept, eject, attempted ejection). When a host accepts an egg outright, it does not suffer rejection costs. However, when it ejects or attempts to eject a cowbird egg, it could damage some of its own eggs. Before we could test this prediction, we had to determine whether the different treatments (natural parasitism, experimental parasitism using real cowbird eggs, experimental parasitism using artificial cowbird eggs) influenced costs. To determine the relationship between the number of host eggs lost (cost = dependent variable) and the different treatments applied (independent variable), we used a generalized linear mixed model (GLMM) fitted to a Poisson distribution with a log link. We used nest identity as a random effect in all models to avoid pseudoreplication because birds were not banded and some individuals may have been retested in multiple years. Finding no difference between treatments ( $P > 0.4$ ) from our GLMM, we evaluated our prediction without treatment as a variable (no influence of treatment on cost) or separately based on the different treatments (treatment influenced cost).

To determine the factors influencing the cost of parasitism, we created a GLMM with different responses (accept, eject, attempted ejection) as well as potential alternative explanations for responses, including variation between years and clutch initiation date (measured by Julian date) as independent variables (cost ~ response + year + date). Responses could vary yearly depending on the likelihood of being parasitized (Davies et al., 1996) and also by clutch initiation date, with hosts being more likely to eject earlier (Lotem et al., 1992) or later in the nesting season (Lang, Bollinger, & Peer, 2014). We also evaluated whether the number of host eggs present at the time of parasitism and the timing of parasitism (i.e. during laying or incubation) influenced cost. Number of host eggs present could influence response because the more host eggs present, the more that could potentially be damaged during ejection attempts. Timing of parasitism could influence ejection because some hosts are less likely to eject when eggs are laid during incubation (Rothstein, 1976). Because of missing values for the two latter factors, we developed an additional GLMM model with a reduced number of observations that included number of eggs and timing as well as any relevant predictors from the model on the full

data set. In both models, we evaluated the influence of each variable by examining its  $\beta$  estimate and 95% confidence intervals (CI), and we considered confidence intervals not containing zero to be influential. We also estimated the predicted responses and SEs from models with influential parameters. We conducted our analysis using the generalized linear mixed-effects models (glmer) in the package lme4 (Bates, Mächler, Bolker, & Walker, 2015) for the R platform (R Core Team, 2014). An additional factor that likely affected the cost of dickcissel response was multiple parasitism. There were too few observations to include in our model, but we include them in our summary statistics.

To analyse nestling mortality in unmanipulated nests in Kansas and Oklahoma, we grouped nests into one of the following parasitism intensity categories: unparasitized nests or nests that contained either one, two, or three or more cowbird nestlings (by definition, the number of cowbird nestlings in nests was determined from cowbirds that hatched, rather than from the number of cowbird eggs in nests). From these data, we estimated dickcissel nestling mortality (1, 0) from the number of nestlings present on the last visit during which the nest was active and evidence of fledging when nests were subsequently found empty (e.g. sufficient age, faecal sacs in nests, parental behaviour proximate to nests and direct observation of fledglings). There was no need to estimate daily nestling mortality rate (Mayfield, 1975) because each nestling was observed from hatching onward. We compared dickcissel nestling mortality (number lost/number hatched per nest) to the parasitism intensity categories using logistic regression (Proc Logistic in SAS, SAS Institute, Cary, NC, U.S.A.). We treated parasitism intensity as a categorical variable because we were interested in the effect of dickcissels raising no cowbirds versus one, two, or three or more cowbird nestlings. As total brood size (number of dickcissel and cowbird nestlings per nest) might be positively correlated with

competition among nestlings and noise that attracts predators, thereby affecting nestling mortality, we included total brood size as a continuous covariate per nest. However, brood size was not substantially correlated (i.e. not collinear) with a continuous score (i.e. 0, 1, 2, 3) of parasitism intensity ( $r = 0.18$ ). Effects of predictor variables were evaluated as above by using  $\beta$  estimates and their 95% CIs. Models with and without the brood size covariate were compared using Akaike's information criterion corrected for small sample sizes ( $AIC_c$ ).

#### Ethical Note

We attempted to minimize disturbance when experimentally parasitizing nests. Experiments were conducted at the nest in <5 min, and when real cowbird eggs were used, they were typically transferred between nests within 30 min. Eggs were placed in containers during transfer to minimize the chances of damage to the egg or developing embryo. To decrease the likelihood of nest desertion due to partial clutch reduction, we never collected more than a single cowbird egg from a host nest. All procedures were approved by the Institutional Animal Care and Use Committee of Western Illinois University (IACUC protocol number 07-56), Illinois Department of Natural Resources (permit number NH 5062) and the U.S. Fish and Wildlife Service (permit number MB122718). Our study followed the ASAB/ABS Guidelines for the use of animals in research.

## RESULTS

### Host Response to Parasitism

Cowbirds parasitized 35.2% of 301 dickcissel nests in Illinois. We found no evidence that the different treatments influenced the



**Figure 1.** Examples of damage resulting from ejection attempts by dickcissels. (a) Dickcissel egg punctured after ejection attempt. (b) Dickcissel egg dented after the host bird's bill bounced off the cowbird egg into one of its own eggs. (c, d) Cowbird and dickcissel eggs found below nests.



number of host eggs lost during the nesting event. Using natural parasitism as the reference category, adding a real cowbird egg ( $\beta = -0.21$ , 95% CI [-0.73 to 0.27]) or an artificial cowbird egg ( $\beta = -0.09$  [-0.63 to 0.42]) had parameter estimates that included zero, indicating that they were not relevant predictors. Dickcissels accepted cowbird eggs at 43.4% ( $N = 59$ ) of the nests, successfully ejected 22.1% ( $N = 30$ ) of cowbird eggs and attempted to eject eggs at 34.6% ( $N = 47$ ) of nests. Of the individuals that attempted to eject, 60% continued to incubate the parasite egg once their eggs were damaged, and in total, 64% of the birds either accepted or continued incubating the parasitic egg after they had damaged their own eggs.

We ran the model with and without artificial cowbird eggs and the results were the same statistically. Our models indicated that dickcissel response to parasitism had a strong influence on the number of host eggs lost (Fig. 1). Compared to the reference category of accepting the egg, both successfully ejecting eggs (full model:  $\beta = 1.64$  [1.35–1.94]; reduced model:  $\beta = 1.48$  [1.17–1.79]) and attempting to eject eggs (full model:  $\beta = 1.23$  [0.95–1.50]; reduced model:  $\beta = 1.35$  [1.07–1.62]) were positive and did not include zero in their 95% CI, indicating that these responses increased the probability that host eggs were lost.

Our model predicted that no host eggs would be lost per nest (SE = 0.12) when dickcissels accepted cowbird eggs. Alternatively, ejecting the cowbird egg would lead to the loss of 49 host eggs and a predicted loss of 1.6 eggs per nest (SE = 0.15), and when attempting to eject the cowbird egg, 56 host eggs would be lost with a predicted loss of 1.2 eggs per nest (SE = 0.13). It is possible that some hosts attempted to eject, but stopped before damaging their eggs; thus, we may have overestimated ejection costs because our evidence was based on visible damage to eggs. Losses included host eggs with puncture holes (Fig. 1a;  $N = 7$  nests), host eggs that were dented, possibly after the host's bill ricocheted off the cowbird egg and onto its own eggs or when cowbird eggs dropped back onto the other eggs during failed ejection attempts (Fig. 1b;  $N = 12$  nests), and host eggs that appeared below the nest usually intact ( $N = 10$  nests; Fig. 1c, d) and sometimes along with cowbird eggs ( $N = 3$  nests). There were also nests that had only cowbird eggs below them ( $N = 4$ ). For those dickcissels that damaged eggs during ejection attempts, it was important to determine whether dented host eggs hatched. Conducting a post hoc analysis we were able to determine the fate of single dented eggs at seven nests, of which 43% hatched. In contrast to the experimental nests, we found no damaged eggs in or below control nests ( $N = 46$ ) and one control nest was deserted. Only one parasitized nest was deserted when no attempts at ejection were made. Mean clutch size in control nests ( $4.03 \pm 0.16$  SE;  $N = 32$ ) was significantly greater than naturally parasitized clutches for which we could determine clutch size ( $2.91 \pm 0.12$  SE;  $N = 75$ ;  $t$  test:  $t_{105} = 5.37$ ,  $P < 0.0001$ ).

Annual variation (year 2:  $\beta = -0.04$  [-0.35 to 0.27]; year 3:  $\beta = -0.01$  [-0.27 to 0.26]) and Julian date ( $\beta = 0.01$  [-0.01 to 0.015]) in the model with the full data set were not relevant predictors of the loss of host eggs. Similarly, in our model with the reduced data set ( $N = 98$  nests), timing ( $\beta = -0.30$  [-0.56 to 0.01]) had little influence on the number of eggs lost. However, as the

number of eggs present at the time of parasitism increased, so did the number of host eggs that were lost ( $\beta = 0.13$  [0.001–0.29]).

Twelve nests were multiply parasitized and we also reparasitized four individuals (Appendix, Table A1). Five individuals successfully ejected both cowbird eggs, one accepted both eggs, whereas the majority of birds were unsuccessful in ejecting both eggs ( $N = 10$ ). We replaced all dickcissel eggs with cowbird eggs at two nests and they were accepted at one nest while the second was deserted (Appendix, Table A1).

### Nestling Mortality

Of 466 dickcissel nests found in Kansas and northeastern Oklahoma, 50.9% were parasitized and 114 were monitored from before or at hatching until nest completion (depredation or fledging). Of these 114 nests, 68 contained no cowbird nestlings, 20 contained one cowbird nestling, 14 contained two cowbird nestlings and 12 contained three or more cowbird nestlings. All dickcissel nestlings fledged in the majority of nests in which any young fledged: 79 of 114 nests fledged young, of which only seven nests were estimated to have fledged fewer host young than the number hatched. In the model including the brood size covariate, dickcissel nestling mortality was not significantly related to brood size ( $\beta = 0.02$  [-0.24 to -0.27]) and this model was inferior ( $AIC_c = 401.7$ ) to the model with parasitism intensity as the only predictor variable ( $AIC_c = 399.6$ ).

In the latter model, dickcissel nestling mortality did not differ between unparasitized nests and nests containing a single cowbird ( $\beta = 0.61$  [-0.05 to 1.26]). Dickcissel nestling mortality in nests with two ( $\beta = 1.39$  [0.57–2.22]) and three or more ( $\beta = 1.24$  [0.32–2.16]) cowbird nestlings was significantly higher than in unparasitized nests (Table 1).

### DISCUSSION

Paradoxically, dickcissels lost the most eggs (1.6) when they successfully ejected the cowbird egg. They lost 1.2 eggs when they attempted to eject the cowbird egg and they lost no eggs when they accepted the cowbird egg. Damage included punctured and dented eggs in parasitized nests, apparently from dickcissel bills ricocheting off the thick-shelled cowbird eggs onto their own eggs (Rohwer & Spaw, 1988; Sealy, 1996), but it is possible some eggs were damaged when the cowbird egg struck them during laying in naturally parasitized nests (López, Fiorini, Ellison, & Peer, 2018). We also found both dickcissel and cowbird eggs below parasitized nests, sometimes intact. Although rejecters typically carry foreign eggs away from the nests (Peer & Sealy, 2004b; Rothstein, 1975a), dickcissels may have had difficulty ejecting cowbird eggs, as has been observed in similarly small-billed cedar waxwings, *Bombycilla cedrorum*, for which cowbird eggs are frequently found below their nests (Rothstein, 1976; see below). We never found eggs below unparasitized control nests, nor were damaged eggs found in control nests. Dickcissels were intolerant of cameras at their nests (see also Rivers, Blundell, Loughin, Peer, & Rothstein, 2013), making

**Table 1**  
Sample sizes of nests and nestlings, mean host brood size and mortality rates of dickcissel nestlings (number that failed to fledge/number of nestlings that hatched, pooled across nests) in nests with 0, 1, 2 or  $\geq 3$  brown-headed cowbird nestlings

| Cowbird nestlings | No. of nests | Dickcissel nestlings | Mean host brood size (SE) | Mortality rate | Host nestling losses <sup>a</sup> |
|-------------------|--------------|----------------------|---------------------------|----------------|-----------------------------------|
| 0                 | 68           | 267                  | 3.926 (0.128)             | 0.225          | 0.882 (0.200)                     |
| 1                 | 20           | 49                   | 2.450 (0.266)             | 0.347          | 0.850 (0.319)                     |
| 2                 | 14           | 26                   | 1.857 (0.177)             | 0.538          | 1.000 (0.234)                     |
| $\geq 3$          | 12           | 20                   | 1.667 (0.320)             | 0.500          | 0.833 (0.271)                     |

<sup>a</sup> Nestling mortality rate multiplied by mean host brood size.

it difficult to document how eggs were ejected. In one case we observed a female using her bill and head to nudge a cowbird egg over the side of the nest (B. D. Peer, personal observation), and such novel means of egg ejection may be more common than previously realized (e.g. De Mársico, Gloag, Ursino, & Reboreda, 2013). In cases where we found intact host eggs below nests, the host may have used this method to remove a cowbird egg and accidentally shoved its own egg over the edge. When we reparasitized nests of four individuals after they ejected a cowbird egg (Appendix, Table A1), they all at least attempted to eject the second parasitic event, and three of the individuals lost or damaged one of their own eggs, indicating that these events were the result of dickcissels responding to cowbird eggs rather than the eggs being damaged by cowbirds. When cowbirds remove host eggs from nests, they fly away with the eggs and sometimes consume them, rather than dropping the eggs below the nests (Sealy, 1992). Cowbirds also destroy eggs by puncturing them when they locate nests that are too advanced for successful parasitism (Arcese, Smith, & Hatch, 1996; Hoover & Robinson, 2007), but we did not find this type of damage at control nests and this cannot explain the intact eggs below nests.

It may seem counterintuitive that there was no difference in rejection costs when using real versus artificial eggs and that the results were the same whether we included artificial eggs or not. However, these results are similar to Antonov et al. (2009), who found no difference in rejection of artificial eggs and real eggs by a small puncture-ejecting cuckoo host, the eastern olivaceous warbler, *Hippolais pallida*.

The presence of a single cowbird nestling did not significantly affect the mortality rate of dickcissel nestlings when compared to unparasitized nests (Table 1). Losses in nests containing two or more cowbird eggs were more substantial, but even nests with three or more cowbird eggs experienced nestling losses similar to those in nests with one cowbird nestling (Table 1). Note that multiple parasitism of dickcissels is uncommon throughout their range (Jaster, Jensen, & Forbes, 2014; Jensen & Cully, 2005; but see Rivers & Peer, 2016). Parasitized clutches had one less egg than unparasitized clutches, presumably due to host egg removal by female cowbirds (Sealy, 1992) or by the host itself after an egg had been struck and damaged by cowbird eggs during laying (López et al., 2018). Consequently, when adding losses from ejection and from host egg removal, either by female cowbirds or by hosts following damage during laying, but no additional costs from nestling losses, individuals that accepted parasitism incurred the lowest costs (1.1 fewer eggs/nestlings), followed by those that attempted to eject cowbird eggs (2.3 fewer eggs/nestlings) and those that successfully ejected cowbird eggs (2.7 fewer eggs/nestlings), which suffered the greatest costs (Table 2). After accounting for the fact that 43% of damaged eggs still hatched, the pattern of losses remained the same (1.1, 1.78, 2.01, respectively; Table 2). Because there was a significant effect of having more than one cowbird nestling on dickcissel nestling mortality, we would expect dickcissels to be more responsive to multiple parasitism and this was the case. Of 16 individuals that were multiply parasitized, 15 either successfully ejected or unsuccessfully tried to eject. However, 10 of these birds were unsuccessful in ejecting both cowbird eggs, which suggests that cowbirds can force acceptance by multiply parasitizing a nest (see also Gloag, Fiorini, Reboreda, & Kacelnik, 2012; Moskat et al., 2009).

The majority of dickcissels (57%) ejected or attempted to eject cowbird eggs, but the costs of raising a single cowbird (nestling mortality) did not differ significantly from that for unparasitized nests, which may explain why most birds (64%) accepted or stopped trying to eject once their eggs were damaged. Why do dickcissels attempt to eject, when based on our estimates,

**Table 2**

Dickcissel response to cowbird eggs, and costs from ejecting a cowbird egg and host egg removal by female cowbirds

| Strategy (% occurrence)  | Rejection cost <sup>a</sup>       | Host egg removal | Total loss                       |
|--------------------------|-----------------------------------|------------------|----------------------------------|
| Accept (43%)             | 0                                 | 1.1              | 1.1                              |
| Attempted ejection (35%) | 1.2 ( <b>0.684</b> ) <sup>b</sup> | 1.1              | 2.3 ( <b>1.78</b> ) <sup>b</sup> |
| Eject (22%)              | 1.6 ( <b>0.912</b> ) <sup>b</sup> | 1.1              | 2.7 ( <b>2.01</b> ) <sup>b</sup> |

<sup>a</sup> Number of eggs lost or damaged.

<sup>b</sup> Values in bold have been adjusted to take into account that 43% of damaged eggs still hatched.

acceptance leads to the lowest cost? One explanation is there are additional costs that we did not measure. These include carryover fitness costs on dickcissel nestlings after sharing a nest with a cowbird and the effects on parents from raising a cowbird (e.g. Payne & Payne, 1998), which could explain why some individuals persisted in trying to eject cowbird eggs despite immediate rejection costs.

Considering the costs of ejecting cowbird eggs, the losses from egg removal by female cowbirds and the damage to host eggs during laying by the parasite, dickcissels should invest more in frontline defences to prevent cowbirds from parasitizing their nests in the first place. Cape bulbuls, *Pycnonotus capensis*, use such a strategy against Jacobin cuckoos, *Clamator jacobinus*. This cuckoo lays very large, thick eggs that are almost impossible to eject, and bulbuls aggressively defend their nests against parasitism (Krüger, 2011). Another alternative would be to reject cowbird nestlings (e.g. Sato, Tokue, Noske, Mikami, & Ueda, 2010), and Hatch (1983) speculated that dickcissels may preferentially feed their young in parasitized nests.

Our results are the first to demonstrate a prohibitive cost of egg ejection in a cowbird host. Rohwer and Spaw (1988) hypothesized that hosts with small bills cannot effectively puncture-eject thick-shelled cowbird eggs and that acceptance is less costly than incurring ejection costs. Female dickcissel bills are 13.8 mm long (Peer & Sealy, 2004a) and the smallest known ejecter species prior to our study was the warbling vireo, *Vireo gilvus*, which has a bill length of 17.6 mm (Sealy, 1996). Warbling vireos puncture-eject or grasp-eject cowbird eggs and lose only 0–0.3 eggs per ejection (Sealy, 1996; Underwood & Sealy, 2011). The effect of having a smaller bill in dickcissels is apparent in that our models predicted a loss between 1.2–1.6 eggs per ejection attempt, and they were not always successful in ejecting the eggs. This is supported by a previous study in which dickcissels ejected a low frequency (11%) of artificial cowbird eggs, but ejected 100% of undersized cowbird eggs that they could more readily grasp-eject (Peer et al., 2000).

The flexibility in dickcissel response to parasitism is in stark contrast to other North American hosts, the majority of which reject or accept nearly 100% of cowbird eggs (Peer & Sealy, 2004a; Rothstein, 1975a). Instead, this acceptance of parasitism is similar to the eastern olivaceous warbler, a cuckoo host that successfully ejects only 12% of cuckoo eggs and eventually accepts 44% of them because of the increased strength of the cuckoo eggs and the greater probability of damaging their own eggs during puncture-ejection (Antonov et al., 2009). However, cuckoos are nestling-ejectors, so the costs of acceptance by the eastern olivaceous warbler are significantly higher than those experienced by dickcissels that accept cowbird eggs. The only other cowbird host known to modify its ejection strategy is the cedar waxwing and it is more likely to accept when parasitized later in the nesting cycle because the cowbird egg is less likely to hatch (Rothstein, 1976). It also has a relatively small bill (19.8 mm; Peer & Sealy, 2004a), albeit larger than the dickcissel's bill, and it damages its own eggs when trying to eject cowbird eggs (Rothstein, 1976). The interactions between

cowbirds and their grassland hosts may be more refined due to the longer period of intense parasitism (Mayfield, 1965; Peer et al., 2000), and the defences exhibited by dickcissels appear to be more sophisticated than those of other cowbird hosts.

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## Appendix

**Table A1**

Outcome at multiply parasitized nests ( $N = 12$ ), nests that we reparasitized ( $N = 4$ ) and nests at which we replaced all dickcissel eggs with real cowbird eggs ( $N = 2$ )

| Nest  | Parasitism <sup>a</sup> | No. cowbird eggs | Response  | Notes                               |
|-------|-------------------------|------------------|---|-------------------------------------|
| 06–3  | 1                       | 2                | Accepted  |                                     |
| 06–50 | 1                       | 2                | Ejected 1, accepted 1   | Lost 1 host egg, damaged 1 host egg |
| 06–57 | 1                       | 2                | Attempted ejection  | Lost 1 host egg                     |
| 06–66 | 1                       | 2                | Ejected 1, accepted 1   | Lost 1 host egg                     |
| 06–73 | 1                       | 2                | Ejected 1, accepted 1   | Lost 2 host eggs                    |
| 07–18 | 1                       | 2                | Ejected both cowbird eggs   | Lost all host eggs                  |
| 07–31 | 1                       | 2                | Attempted ejection  | Damaged 1 host egg                  |
| 07–47 | 1                       | 2                | Ejected both cowbird eggs   | Lost all host eggs                  |
| 07–70 | 1                       | 2                | Attempted ejection  | Damaged 1 host egg                  |
| 07–87 | 1                       | 2                | Attempted ejection  | Damaged 1 host egg                  |
| 08–3  | 1                       | 2                | Ejected 1, accepted 1   | Lost 1 host egg                     |
| 08–44 | 1                       | 3                | Attempted ejection  | Lost 1 host egg                     |
| 06–1  | 1, 2                    | 2                | Ejected both real cowbird eggs                                      | Lost 1 host egg                     |
| 06–40 | 1, 2                    | 2                | Ejected artificial cowbird egg; attempted to eject real cowbird egg | Damaged 1 host egg                  |
| 06–86 | 1, 2                    | 2                | Ejected both real cowbird eggs                                      | Damaged 1 host egg                  |
| 07–3  | 1, 2                    | 2                | Ejected artificial and real cowbird eggs                            |                                     |
| 07–63 | 2                       | 4 <sup>b</sup>   | Deserted  |                                     |
| 07–83 | 2                       | 3 <sup>b</sup>   | Accepted  |                                     |

<sup>a</sup> 1: natural parasitism; 2: experimental parasitism.

<sup>b</sup> All host eggs were replaced with real cowbird eggs.