



Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis



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Parasites show a wide variety of strategies to maximize the successful transmission of themselves and/or their offspring, by exploiting hosts. One such strategy occurs when parasites manipulate host behaviour in a way that increases their probability of transmission to an uninfected host. Here, we examine whether brood parasitic brown-headed cowbirds, *Molothrus ater*, attack and cause nest failure in late-stage, and hence, inappropriate host nests, which theory suggests they may do to parasitize the replacement nests at an opportune time, effectively manipulating their host's reproductive behaviour and improving their own transmission. Critical to this 'farming' hypothesis, cowbirds must be attuned to the reproductive stage of their host and act accordingly by destroying nonparasitized clutches they find late in the nesting cycle. We conducted a series of experimental manipulations in which we presented captive cowbirds with nests simulating early and late stages. We found that cowbirds caused significantly greater destruction in the late-stage nests. Moreover, our results suggest that cowbirds are capable of using both direct assessment and absolute egg number to assess which clutches to destroy. Corroborating our findings in the laboratory, 10 years of field data show that cowbirds significantly increase the intensity of their attacks (i.e. the proportion of the clutch destroyed) on nonparasitized host nests as the nesting cycle progresses; however, we found no such trend for parasitized host nests. These results indicate that cowbirds evaluate the reproductive stage of their hosts using multiple mechanisms and use this information to vary the intensity of their attacks.

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Parasites, of all sorts, show a wide variety of strategies to maximize the successful transmission of themselves and/or their offspring, by exploiting typically unrelated hosts (Poulin, 2007). One such strategy occurs when parasites manipulate host behaviour in a way that increases their probability of transmission to an uninfected host (Holmes & Bethel, 1972; Poulin, 2000). Examples of parasites altering host behaviour abound, and the effects may be dramatic and often focus on host reproductive behaviour (Moore, 2002). For instance, rodents infected with *Toxoplasma gondii* appear less 'fearful' of the parasite's definitive cat host (Berdoy, Webster, & Macdonald, 2000) and may even seem more sexually attractive to uninfected members of the opposite sex (Vyas, 2013). Host behavioural changes such as these may be considered an adaptive extended phenotype of the parasite (Dawkins, 1982),

when the behavioural change is actually caused by the parasite and can be shown to increase the probability of transmission (reviewed in: Moore, 2002; Poulin, 1995; Thomas, Adamo, & Moore, 2005). Also critical, is the need to identify the mechanisms involved in the behavioural change in order to understand the complexity and sophistication of the supposed manipulation (Nickol, 2005; Thomas et al., 2005). Brood-parasitic birds are functionally indistinguishable from conventional parasites and pathogens in that they may reduce the reproductive success of infected hosts (Davies, 2000; Hauber, 2003; Kilner, 2005; Ortega, 1998; Smith, Tait, & Zanette, 2002) and their transmission success is wholly dependent on their ability to encounter hosts suitable for parasitism during the infective stage (i.e. during egg laying/early incubation). Nevertheless, instances of adult brood parasites manipulating host behaviour in order to increase the probability of infection have rarely been considered (but see Hoover & Robinson, 2007; Ponton, Biron, Moore, Møller, & Thomas, 2006; Soler, Soler, Martinez, & Møller, 1995).

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Obligate avian brood parasites, such as cowbirds and cuckoos, lay their eggs in the nests of other species ('hosts') whereupon the host parents provide all parental care for the parasitic young (Davies, 2000). To successfully 'infect' a host, a brood parasite must be attuned to its hosts' reproductive stage as there is only a narrow window of time during egg laying and early incubation in which parasitism will be effective (Fiorini, Tuero, & Rebores, 2009). Some brood parasites also attack host nests, destroying eggs/nestlings and causing nest failure of their potential hosts (Arcese, Smith, & Hatch, 1996; Peer & Sealy, 1999; Soler et al., 1995). Such behaviour is perplexing as it appears to be a superficial waste of a laying opportunity on the one hand, but these predatory habits could actually increase the probability of successful transmission (Arcese et al., 1996). The farming hypothesis suggests that brood parasites will destroy, or 'farm', host nests found too late in the nesting cycle to be suitable for parasitism, thereby manipulating those hosts into starting a new reproductive cycle prematurely, ultimately creating future opportunities to parasitize the host's re-nesting attempts (Arcese, Smith, Hochachka, Rogers, & Ludwig, 1992; Arcese et al., 1996). As long as the parasite focuses its attacks on late-stage nests no laying opportunity is wasted. Hence, as with parasitic egg laying, 'farming' behaviour requires that the parasite can assess the host's reproductive stage and acts accordingly by destroying late-stage nests.

We investigated the predatory behaviour of the brood-parasitic brown-headed cowbird, *Molothrus ater* (hereafter cowbird) to determine whether they were consistent with what would be expected from the farming hypothesis. Cowbirds are common throughout North America and are extreme host generalists, successfully parasitizing over 100 species of passerines (Lowther, 1993). To date, most studies of the farming hypothesis have focused on whether or not cowbirds are substantial nest predators because this is one general condition that would be necessary if farming were occurring. While several lines of evidence indicate that they are (Arcese et al., 1996; Clotfelter & Yasukawa, 1999; Granfors, Pietz, & Joyal, 2001; Hoover & Robinson, 2007), the critical prediction that cowbirds focus their attacks on late-stage host nests that are no longer suitable for parasitism remains untested. Moreover, the recognition mechanisms that cowbirds use to discriminate early- from late-stage nests before deciding to attack are largely unknown (King, 1979). Cowbirds are adept at finding hosts and appear to be capable of discriminating appropriate from inappropriate nests when deciding whether to lay in a nest (White, Ho, & Freed-Brown, 2009; White, Ho, de los Santos, & Godoy, 2007). If cowbirds are indeed 'farming' their hosts, then it makes logical sense that the parasite must be attuned to the host's reproductive cycle and use this knowledge to assess whether or not to destroy nests.

Cowbirds typically cause nest failure by either puncturing or removing enough eggs to cause the host parents to abandon. Beyond farming, other hypotheses have been proposed to explain egg removal or egg puncture by cowbirds (Hoover & Robinson, 2007; Peer, 2000; Sealy, 1992). For example, female cowbirds are known to occasionally consume the eggs they destroy (Sealy, 1992). Nutrition does not appear to be the primary motivator for attacking nests, however, as most documented incidents do not involve the cowbird eating any nest contents (Granfors et al., 2001; Scott, Weatherhead, & Ankney, 1992; Sealy, 1992). In cowbird parasitized nests, removal of host eggs may enhance the incubation efficiency of the parasitic egg (incubation efficiency hypothesis: Peer & Bollinger, 2000) or reduce future competition for a parasitic nestling (competition reduction hypothesis: Carter, 1986; Llambias, Ferretti, & Rebores, 2006). Also in parasitized nests, if a host rejects the parasitic egg, the parasite may retaliate by destroying enough of

the nest contents to cause complete failure (mafia hypothesis: Hoover & Robinson, 2007). The incubation efficiency, competition reduction and mafia hypotheses do not, however, explain attacks on nonparasitized nests, which are the focus of the farming hypothesis and our study.

The most direct and reliable way a cowbird could gauge the developmental stage of a host nest is by puncturing a portion of the clutch (Massoni & Rebores, 1999). Conceivably, the degree of development of a single host embryo could easily be evaluated in this way and such information could be used when deciding whether or not to destroy the clutch. Other indirect methods of evaluating nest stage include attending to cues (e.g. absolute egg number) that are indicative of a clutch being complete and most likely late stage (White et al., 2009, 2007). Here, we report the results from a series of experiments in the laboratory demonstrating that cowbirds use both direct and indirect methods to gauge host egg stage and are more likely to destroy evidently later-stage eggs. These experimental results in the laboratory are corroborated by evidence from 10 years of field data suggesting that cowbirds preferentially cause late-stage nests to fail likely in an attempt to 'farm' their hosts.

METHODS

Experimental Procedures

We performed a series of 'cafeteria style' choice experiments designed to test whether female cowbirds preferentially destroy late-stage nests that would no longer be suitable for parasitism (as per the farming hypothesis). We also evaluated whether cowbirds use direct and/or indirect methods of assessing the age of host eggs. Specifically, we examined the tendency of females to approach and attack artificial nests that contained eggs of different developmental stages or different numbers of eggs. We assessed whether female cowbirds would preferentially attack nests that simulated late- versus early-stage nests by comparing nests containing (1) highly developed eggs versus freshly laid and undeveloped eggs, (2) different numbers of eggs, permitting us to determine whether absolute or relative number is important and (3) varying numbers of eggs across days versus a continual 'full' clutch.

We captured 58 adult cowbirds (40 female and 18 male) using mist nets and funnel traps baited with cracked corn at Long Point Bird Observatory and Ruthvin Park Banding Station in Ontario during April 2012. Cowbirds were transported to the University of Western Ontario, Canada, colour-banded for individual recognition and housed in four large outdoor cages at the Advanced Facility for Avian Research (AFAR). Birds were fed a modified Bronx Zoo diet for omnivorous birds daily (see White et al., 2007) and had ad libitum access to white millet, canary seed mix, crushed oyster shells and vitamin-treated water. In order for cowbirds to regularly lay eggs in captivity, they require spacious outdoor aviaries much larger than the cages our birds were housed in (White et al., 2009, 2007). However, we assumed that our birds were in breeding condition because eggs were occasionally found (approximately two per week) within the cages, and males and females continued to perform breeding displays throughout the duration of the study.

Before each trial for each experiment, individual birds were transferred to an outdoor flight chamber and left for 24 h to habituate. We randomly selected a subset of females from the captive population for each experiment, and subjects used multiple times had a minimum 5-day interval between trials (mean \pm SE = 18 \pm 1.7 days). Flight chambers contained natural perches situated across one wall, along with food and water.

Immediately before each trial, two artificial open-cup nests containing different egg stimuli (see below for details) were placed in either corner of the wall opposite the perches approximately 1 m from the ground. A short perch was positioned proximate (within 10 cm) to each nest, to facilitate investigation. The nests were constructed from raffia and white glue shaped around a tennis ball. Trials commenced as soon as the researcher left the flight chamber and lasted for 20 min. All trials were videorecorded in the absence of an observer. Generally we found that female cowbirds readily attacked experimental nests and usually punctured or removed their first egg within the first 5 min of a trial (mean \pm SE = 199.3 \pm 28.9 s).

For each experiment we recorded and compared three possible measures of a cowbird's intent to cause a nest to fail. First, we compared the incidence of attack (i.e. total number of nests in which one or more eggs were destroyed) and the total number of visits (bird lands on nearby perch: following White et al., 2007) to each nest type. Second, we compared the overall attack intensity (proportion of eggs destroyed per nest) for each nest type. We reasoned that the proportion of host eggs removed or punctured during an attack was a reasonable proxy for attack intensity and a bird's intent to cause failure (see Field Procedures below for justification). Third, we compared the number of incidents in which the entire nest contents were destroyed. This is the most unambiguous measure of intent to cause nest destruction, which is a necessary element of the farming hypothesis.

Attacks and Developmental Stage

The most direct way that cowbirds may assess the viability of a nest is by attending to the developmental stage of eggs (White et al., 2009, 2007). For example, shiny cowbirds, *Molothrus bonariensis*, may gain valuable information in deciding whether or not to parasitize a nest by puncturing a host egg to assess embryonic development of the entire clutch (test-incubation hypothesis; Massoni & Reboreda, 1999). When 'farming' their hosts, brown-headed cowbirds could use such information when deciding whether to cause nest failure. We predicted that cowbirds puncture few eggs if nests are in the early incubation stage and that they puncture most or all of the eggs if nests are in the late incubation stage. To assess this possibility, 26 females were presented with two nests, one containing eggs collected when freshly laid (0–1 days incubated) and the other containing eggs collected when highly developed (11–12 days incubated). Each nest contained three eggs, thereby allowing us to isolate the effect of egg development from clutch size as a cue to nest age.

Eggs were collected from the nests of red-winged blackbirds, *Agelaius phoeniceus*, which are commonly parasitized by cowbirds and likely suffer from cowbird predation (Clotfelter & Yasukawa, 1999). After collection, eggs were stored at 4 °C for a minimum of 10 days, where as little as 4 h of cooling would have ensured effective and humane euthanization (Leary et al., 2013). All eggs were returned to ambient temperature prior to presentation.

Red-winged blackbirds are among the most abundant species of bird in North America (Beletsky, 1996; Yasukawa & Searcy, 1995) and are not protected under either federal or provincial law because they are considered agricultural pests (Migratory Birds Convention Act, 1994). Also, the Canadian Council on Animal Care places eggs in the lowest category of invasiveness for wildlife studies (Category A: CCAC 2010). Even so, to minimize the impact on blackbird reproduction, we collected one egg per nest and only enough eggs to ensure a minimum sample size of 10 for this experiment (30 undeveloped, 30 developed eggs). Eggs left undamaged in trials were reused in subsequent trials so that we could

maximize the total number of trials we could carry out without requiring collection of additional eggs ($N = 26$). In accordance with Canadian law and guidelines outlined by the Canadian Council for Animal Care, these protocols were approved by the University of Western Ontario Animal Care Committee (Protocol Number: 2010-005).

Attacks and Egg Number

The number of eggs in a nest may indirectly signal the approximate age of a nest to cowbirds (White et al., 2007). Whereas females prefer to parasitize nests with a clutch size indicative of a nest at the egg-laying stage (King, 1979; Trine, 2000; White et al., 2007), we expect that they should preferentially attack nests that have a number of eggs representative of a complete clutch. The typical clutch size for cowbird hosts ranges from three to six eggs, but is most commonly four, and most hosts begin incubation after laying the last or second-to-last egg (Friedmann, Kiff, & Rothstein, 1977; Terres, 1980). Therefore, we presented 26 females with a nest containing four unfertilized yellow canary, *Serinus flaviventris*, eggs (21.1 \times 14.3 mm), simulating a complete clutch, and a nest containing two canary eggs, simulating an early nest that was still in the egg-laying stage (King, 1979; White et al., 2007).

While the absolute number of eggs in a nest may indicate approximate incubation timing, it is possible that cowbirds may simply attend to the relative number of eggs and target a larger clutch. To confirm whether cowbirds respond to absolute clutch size and to rule out a possible effect of our experimental manipulation, we presented 20 females simultaneously with a nest containing six eggs and a nest containing four eggs. In this experiment both nests contained a number of eggs that would typically represent the completion of egg laying, although a two-egg difference between treatments was maintained. If cowbirds do preferentially attack nests late in the incubation stage, we would not expect to see any behavioural differences between these two nest types because information on their relative developmental stage could not be gleaned from egg number.

Attacks and Changing Number of Eggs

Cowbirds are known to evaluate a nest's suitability for parasitism by monitoring whether eggs appear across multiple days and perceive nests with an increasing clutch as being suitable for parasitism (White et al., 2009). This could allow females to better synchronize their laying with that of their hosts because a nest with a changing number of eggs would always be in the egg-laying stage. A clutch size that could be indicative of completion of laying (i.e. which remains constant in size across days) meanwhile would simulate complete clutches where incubation has already commenced. To determine whether the rate at which eggs are added to a nest across days affects the readiness of female cowbirds to attack, we performed an experiment similar to that described by White et al. (2009). Two nests were set up containing model plasticine eggs. Twenty females were presented with a nest containing two model eggs and a nest containing four model eggs. The initial set-up mirrored experiment 1, but here each nest was wrapped in a protective ball (\approx 25 cm diameter) of 1.25 cm chicken wire mesh. This prevented the cowbirds from puncturing or removing any eggs, while still allowing them to approach and investigate. The following morning a third egg was added to the two-egg clutch. On the third morning a fourth egg was added to the three-egg clutch and the chicken-wire ball was removed from both nests. Thus, trials involved a choice between two nests containing four eggs each: one nest with a changing number of eggs and one with a constant clutch size.

Field Procedures

To complement our experiments using captive birds we assessed whether wild cowbirds tailor their attacks depending on their hosts' reproductive stage. We tested for a positive correlation between the timing of a cowbird attack and the proportion of host eggs destroyed for nonparasitized nests and again for parasitized nests. Based on the farming hypothesis, we expected that the overall attack intensity (proportion of host clutch destroyed) of cowbirds would increase as the nesting cycle progressed for nonparasitized nests only, as explained in the introduction.

We monitored parasitism and predation on 1035 song sparrow nests, *Melospiza melodia*, resident near Victoria, British Columbia, Canada over 10 years (2000–2007, 2009–2010). Song sparrows make an ideal species for studying possible farming behaviour as they are a favourite cowbird host (Woolfenden, Gibbs, Sealy, & McMaster, 2003), do not reject cowbird eggs, and have been the subject of intensive study in the context of parasitism and predation in and around our study sites (Smith et al., 2002; Smith, Taitt, Zanette, & Myers-Smith, 2003; Zanette & Clinchy, 2010; Zanette, Haydon, Smith, Taitt, & Clinchy, 2007). Song sparrows typically lay clutches of three to four eggs and begin incubation after laying their penultimate eggs. A cowbird egg will only receive enough incubation to hatch if laid before day 7 following the laying of the first sparrow egg (Swan, Zanette, & Clinchy, n.d.). Therefore nests were considered inappropriate for parasitism following this time. Other specific details regarding study sites, song sparrows and methods used to find and monitor nests can be found elsewhere (Zanette, Clinchy, & Smith, 2006; Zanette, Smith, van Oort, & Clinchy, 2003). Migratory brown-headed cowbirds begin parasitizing nests in the area in late April (mean \pm SE = 28 April \pm 2.7 days) and lay their last egg by mid-July (mean \pm SE = 8 July \pm 1.6 days). Discounting nests that failed or fledged before cowbirds arrived in the spring, 17–78% of nests were parasitized (contained at least one cowbird egg) in a given year (mean \pm SE = 48.3 \pm 5.3%).

Using behavioural cues from the parents (Zanette et al., 2006), we located nests that were monitored regularly (every 1–4 days) until the nests failed or fledged. During each visit we recorded the number of host and cowbird eggs present in the nest. We candled eggs to determine age or backdated from hatch day. During each visit we also recorded the number of eggs found punctured or removed from the nest. From 2004 to 2010 we monitored 90 song sparrow nests at our study sites using a custom video surveillance system (see Zanette, White, Allen, & Clinchy, 2011 for camera details). We found that 48 of these nests failed as a result of a predator attack and that cowbirds were the number one predator, responsible for 35% of all nest failures (Swan, Zanette, & Clinchy, n.d.). Our video records show that cowbirds either removed all eggs or left punctured eggs in the nest. No other predator was found to leave punctured eggs in our study site. We therefore reasoned that a cowbird had attacked a nest whenever (1) one or more eggs were found punctured in or around the nest, (2) a researcher directly observed a cowbird attacking a nest or (3) a cowbird attack was captured on camera. Under these criteria, we estimate that 74 nonparasitized nests and 62 parasitized nests were attacked by a cowbird. This is likely a conservative estimate of the actual number of cowbird attacks because we did not include nests where we could not definitively assign a cowbird as the predator (i.e. nest contents missing; 217 nests).

Statistical Analyses

For our manipulations in the laboratory, the total number of nests attacked was compared using a Fisher's exact test. The number of visits to each nest type, whether the nest was attacked

or not, was compared using a Wilcoxon signed-ranks test. The proportion of eggs destroyed in each nest type was also compared using a Wilcoxon signed-ranks test. A Fisher's exact test was used to compare the number of attacks that resulted in complete destruction (i.e. those that would have certainly failed) and those that suffered only partial destruction for each nest type. Trials were omitted from all analyses whenever an individual did not puncture or remove at least one egg from either nest. Whenever one or more eggs were destroyed in a nest, we included that trial in all pertinent analyses. For our field data, we used a Spearman rank correlation to compare the proportion of eggs destroyed with the timing of cowbird attack relative to when the first host egg was laid for both parasitized and nonparasitized nests.

RESULTS

Attacks and Developmental Stage

Consistent with the prediction from the farming hypothesis that cowbirds preferentially cause late-stage nests to fail, cowbirds were more likely to destroy all three eggs when they attacked late-stage nests but only one egg when they attacked early-stage nests (Fisher's exact test: $P = 0.02$). Of the 12 attacks on late-stage nests, 66%, 17% and 17% of cases involved the destruction of three, two and one egg, respectively, whereas attacks on early-stage nests showed the opposite pattern (0%, 11% and 89%, $N = 9$). Cowbirds destroyed a significantly greater proportion of eggs in nests containing developed late-stage eggs than in nests containing undeveloped early-stage eggs (Wilcoxon signed-ranks test: $W = 76$, $P = 0.014$; Fig. 1). There was no significant difference in the overall incidence of attacks (Fisher's exact: early-incubation nests = 35%, late-incubation nests = 46%, $P = 0.48$) and cowbirds did not visit late-incubation stage (mean \pm SE = 3.65 \pm 1.24, median = 4, range 0–20) and early-incubation stage (mean \pm SE = 1.35 \pm 0.21, median = 1, range 0–3) nests a significantly different number of times (Wilcoxon signed-ranks test: $W = 105$, $P = 0.16$). We excluded nine of the 26 trials (35%) from analyses because these cowbirds did not destroy any eggs.

Attacks and Egg Number

Consistent with cowbirds using absolute egg number to indirectly determine nest stage, we found that cowbirds destroyed over two times the proportion of eggs in four-egg nests than in two-egg

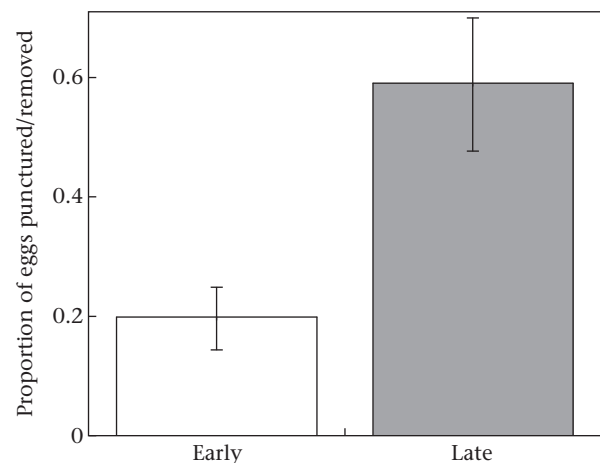


Figure 1. The proportion of eggs punctured or removed from experimental nests containing developed late-incubation stage eggs and nests containing undeveloped early-incubation stage eggs.

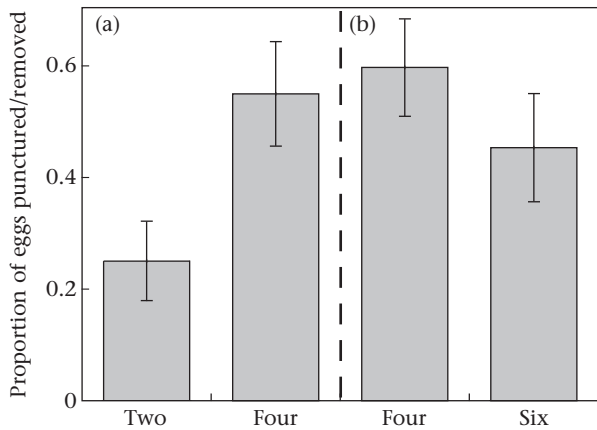


Figure 2. The proportion of eggs punctured or removed from experimental nests containing (a) four-egg and two-egg clutches and (b) six-egg and four-egg clutches.

nests ($W = 112.5$, $P = 0.0136$; Fig. 2a). Out of the 15 four-egg nests attacked, 40% suffered complete destruction and 27%, 20%, 13% had three, two and one eggs destroyed, respectively. Attacks on two-egg nests meanwhile involved complete destruction 11% of the time and destruction of one egg 89% of the time ($N = 9$). Cowbirds did not attack four-egg nests significantly more often than two-egg nests (four-egg = 58%, two-egg = 35%, $P = 0.164$). There was also no significant difference in the number of visits between the two nest types (two-egg: mean \pm SE = 3.76 ± 1.31 , median = 2, range 0–36; four-egg: mean \pm SE = 2.00 ± 0.26 , median = 1, range 0–7; $W = 298.5$, $P = 0.79$). Six of the 26 trials (23%) were excluded from analyses because no eggs were destroyed.

Corroborating that cowbirds attend to the absolute number of eggs in a clutch, when we increased the clutch size so that both treatments simulated complete clutches although a two-egg difference was maintained (four eggs in one nest and six eggs in the other), we found no significant difference in the proportion of eggs destroyed ($W = 130$, $P = 0.31$; Fig. 2b). Moreover, both nest types were attacked an equal number of times ($N = 15$ of 26, $P = 1.00$) and visits to both nests were not significantly different (six-egg: mean \pm SE = 3.47 ± 0.52 , median = 3, range 0–10; four-egg: mean \pm SE = 3.84 ± 0.70 , median = 4, range 0–7; $W = 182.5$, $P = 0.96$). No eggs were destroyed in two out of 20 trials (10%) and were therefore excluded from analyses.

Attacks and Changing Number of Eggs

We found no evidence that cowbirds used the change in clutch size to indirectly evaluate nest stage. There was no significant difference in the proportion of eggs destroyed regardless of whether the clutch size changed on a daily basis or remained constant ($W = 99.5$, $P = 0.96$). Furthermore, there was no difference in the number of attacks that resulted in complete destruction and those that did not ($P = 1.00$). Attacks on nests with a changing clutch number resulted in four eggs, three eggs, two eggs and one egg being destroyed 36%, 9%, 18% and 36% of the time, respectively. Attacks on nests with a constant clutch number ended in destruction of four eggs, three eggs, two eggs and one egg 30%, 50%, 0% and 20% of the time, respectively. There were also no significant differences between treatments in the overall incidence of attacks (constant = 50%, changing = 55%, $P = 1.00$), or the number of visits (constant: mean \pm SE = 2.70 ± 0.744 , median = 3, range 0–14; changing: mean \pm SE = 3.10 ± 0.864 , median = 3, range 0–11; $W = 194.5$, $P = 0.89$). Six out of 20 trials (30%) were omitted from analyses because no eggs were destroyed.

Wild Cowbird Predation

Based on our 10-year data set concerning song sparrow nests in the wild, we found that cowbird attack effort increased significantly as the nesting cycle progressed on nonparasitized nests (Spearman rank correlation: $r_s = 0.35$; $F_{1,72} = 11.08$, $P = 0.001$; Fig. 3), with no significant effect for parasitized nests ($r_s = 0.07$; $F_{1,60} = 0.26$, $P = 0.61$; Fig. 4).

DISCUSSION

Our experiments in the laboratory and our long-term field data demonstrate that destruction of host nests by brood parasitic cowbirds is attuned to their host's reproductive cycle, which would be expected from the farming hypothesis. Our laboratory experiments demonstrate that cowbirds use the most direct and straightforward method of assessing host egg stage (i.e. egg puncture) in addition to using indirect means such as evaluating absolute clutch size. These manipulations confirm that cowbirds intensely attack nests simulating a late-incubation stage by destroying all or a large proportion of eggs, but destroy few eggs from nests simulating an early-incubation stage. These results are supported by our field data showing that the proportion of eggs destroyed by cowbirds in a nonparasitized clutch significantly increases as the nesting cycle progresses.

The underlying mechanisms that we report in our study match some of the criteria necessary to conclude that farming behaviour is adaptive for the parasite (Moore, 2002; Poulin, 1995; Thomas et al., 2005). First, in order for farming to enhance their probability of transmission, cowbirds need to direct intense attacks towards nests no longer suitable for parasitism, which is what our manipulations show. Second, host nests in the wild are more likely to fail as cowbird attack intensity increases, demonstrating that the hosts' behavioural change is unambiguously caused by the parasite. Last, our results suggest that cowbirds are capable of using both direct and more indirect and, thus, potentially more sophisticated mechanisms, to discriminate among nests. The fact that these parasites use multiple methods of discrimination reinforces that farming behaviour is not a random act and instead could be an active attempt to manipulate host reproductive behaviour. However, to complete the picture, one would need to demonstrate that farming improves the probability of transmission if and when the cowbird returns to parasitize the subsequent re-nesting attempts

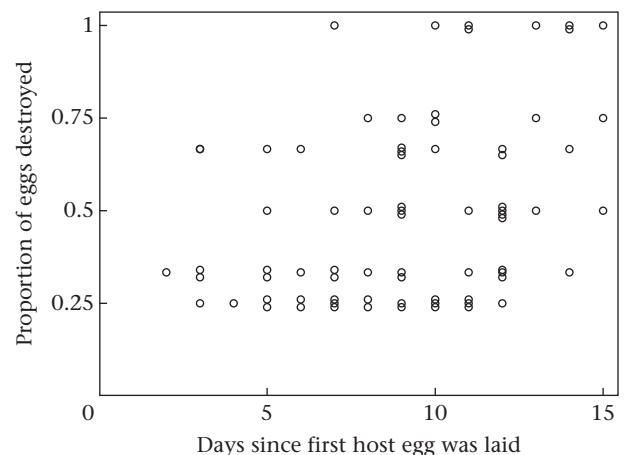


Figure 3. The proportion of eggs killed by cowbirds in nonparasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest ($N = 74$). Points are offset slightly where overlap occurs.

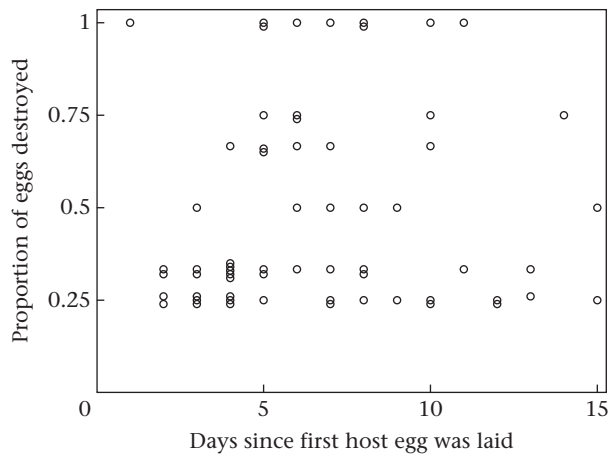


Figure 4. The proportion of eggs killed by cowbirds in parasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest ($N = 62$). Points are offset slightly where overlap occurs.

(Hoover & Robinson, 2007) and/or if cowbird-induced nest failure simply increases the proportion of suitable to unsuitable nests available for parasitism throughout the season. The relative benefits of either strategy should be the focus of future studies.

Puncturing an egg in order to estimate development is likely among the most reliable and direct of methods for determining nest age, and we have demonstrated that cowbirds vary the intensity of their attacks based on this developmental cue. Interestingly, the closely related shiny cowbird has been reported to puncture host eggs to gauge embryonic development to avoid laying in late-incubation stage nests, but that species apparently does not farm their hosts (Massoni & Reboreda, 1999). Whether brown-headed cowbirds also puncture to optimize their timing of parasitism is unknown. The birds in our experiments also appear able to assess the age of a clutch indirectly by attending to absolute clutch size. Our results are consistent with those of White et al. (2007), who found that cowbirds preferentially parasitize nests containing three eggs over nests with one egg, but show no behavioural differences between nests with three versus six eggs. Accurately estimating nest age is important for both parasitism and farming behaviour, and cowbirds appear to use a similar method of doing so in both contexts. Using quantitative information is not unusual for birds (e.g. food hoarding; Hunt, Low, & Burns, 2008) and has been reported for other brood parasites (Odell & Eadie, 2010). Notwithstanding, we cannot yet be certain of a cowbird's numerical competency. Cowbirds, for example, could count individual eggs (e.g. Lyon, 2003) or attend to the volume of eggs in a nest.

White et al. (2009) previously demonstrated that cowbirds attend to the changing number of eggs in a nest across days when deciding to parasitize. We found no indication that the same information is used when attacking nests. We found that cowbirds attacked a similar proportion of eggs in nests with changing clutch size as in nests with constant clutch size. These apparently contrasting results may be due to methodological differences between studies. However, a more compelling explanation is that cowbirds simply do not monitor nests across days when farming hosts. It is reasonable to assume that inaccurately gauging the age of a host nest would be a more costly venture when laying in a nest than farming it, because the former would lead to the mortality of the parasite's offspring. Cowbirds often observe nests over multiple days prior to parasitizing them (Clotfelter, 1998; White et al., 2009) and the extra effort may ensure that parasitism is not mishandled. By contrast, it is conceivable that relying on quick and easy methods

of determining nest stage, such as egg puncture, may yield more efficient farming results.

In our experiments we tested three methods by which cowbirds may assess host reproductive stage in relation to farming, based on established methods used in relation to parasitism (Massoni & Reboreda, 1999; White et al., 2009, 2007). These are not the only methods cowbirds use to evaluate nest stage, however, and various authors have proposed a plethora of different means (e.g. egg temperature, host activity; Banks & Martin, 2001). Our laboratory results demonstrate that cowbirds evaluate nest stage by at least two means, and our field results corroborate that they destroy nests depending on host reproductive stage. Which method they use in the field will of course be governed by the set of cues available and likely requires substantial cognitive processing. Our field and laboratory data were collected using different populations of cowbirds and, while not the focus of this research, future studies comparing nest predation of different populations may yield valuable results. For example, farming behaviour may become less necessary in areas where the density of host nests is high.

Interactions between parasites and their hosts are often cited as textbook examples of an evolutionary arms race (e.g. Alcock, 2005; Krebs, 2009), and several studies in the last decade point to remarkable complexity in these interactions, in particular among brood parasites and their hosts (e.g. Hoover & Robinson, 2007; Kilner, Madden, & Hauber, 2004; Pagnucco, Zanette, Clinchy, & Leonard, 2008; Stoddard & Stevens, 2010; Zanette & Clinchy, 2010). Cowbirds clearly do destroy host nests, and evident farming by brown-headed cowbirds is arguably among the most striking examples of a brood parasite manipulating its host. The results of our study are likely applicable to many host–parasite systems as they illustrate the need for understanding how parasites interact with their hosts. Indeed, by ignoring the costs of host farming in our system, the demographic significance of the parasite may be substantially undervalued.

In summary, our results in the laboratory and field clearly show that cowbirds do discriminate among nests and adjust the intensity of their attacks depending on the perceived age of nonparasitized nests. Such effects are consistent with expectations from the farming hypothesis in that cowbirds seem to selectively cause failure in late-stage nests that are no longer suitable for parasitism. Moreover, we show that cowbirds use multiple methods for discriminating among early- and late-stage host nests, both directly by puncturing eggs and indirectly by monitoring absolute egg number in a clutch.

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