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## ANTIPARASITIC BEHAVIORS OF RED-WINGED BLACKBIRDS (*AGELAIUS PHOENICEUS*) IN RESPONSE TO SIMULATED BROWN-HEADED COWBIRDS (*MOLOTHRUS ATER*): FURTHER TESTS OF THE FRONTLOADED PARASITE-DEFENSE HYPOTHESIS

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**ABSTRACT.**—The Red-winged Blackbird (*Agelaius phoeniceus*; redwing) is a commonly used acceptor host species that incubates eggs and cares for nestlings and fledglings of the obligate brood parasitic Brown-headed Cowbird (*Molothrus ater*; cowbird). This host species, however, may reduce the risk of parasitism with a frontloaded antiparasite strategy in which it attacks parasites that approach active host nests. To test this frontloaded parasite-defense hypothesis (FPDH), we presented taxidermic models of a female Northern Cardinal (*Cardinalis cardinalis*), which represents no threat to redwings, a male cowbird, which cannot lay a parasitic egg, and a female cowbird, together with species- and sex-specific vocalization playbacks for 5 min. We conducted these presentations at 25 active redwing nests at Newark Road Prairie in south-central Rock County, Wisconsin, USA, where 18% of redwing nests were parasitized by cowbirds in 2015. As predicted by the FPDH, the female cowbird mount elicited the most aggressive responses and the female cardinal mount the least aggressive, as measured by number of times more than one male redwing responded and number of times the male host attacked the mount, and by Principal Component Analyses yielding the highest redwing aggressive behavior and intimidation scores. Contrary to the predictions of FPDH regarding the success of nest defense behaviors, male redwings responding at naturally parasitized nests were significantly more likely to attack the mount than males with nests that were not parasitized, although our sample size was small. We also compared our results with those of a study using the same methods in New York State where cowbird parasitism was rare. Redwings in Wisconsin were more aggressive toward the female cowbird mount than redwings in New York. Red-winged Blackbirds appear to frontload their antiparasite defenses, but the success of those defenses depends on individual and population-level experience with parasites. Received 20 October 2015. Accepted 21 January 2016.

**Key words:** *Agelaius phoeniceus*, antiparasite behavior, brood parasitism, Brown-headed Cowbird, *Molothrus ater*, Red-winged Blackbird.

About 1% of bird species are obligate brood parasites, whose reproductive strategy depends exclusively on host species to build nests, incubate eggs, and care for nestlings and fledglings (Davies

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2000). The interactions of brood parasites and their hosts are a highly studied model system of co-evolution because of the tractability of reciprocal adaptations between hosts and parasites across different evolutionary timescales (Rothstein 1990). In these model systems, natural selection favors adaptations of the parasite that increase its ability to reproduce, including the ability to locate suitable host nests, place eggs in those nests, and ensure that the host parent will care for the parasitic young (Moskát et al. 2006). However, because of the fitness disadvantages of parasite eggs and nestlings for the host, natural selection also favors adaptations of the host that increase its ability to avoid being parasitized, including placing nests in inaccessible locations (Hauber 2001, Hoover and Robinson 2007) or in dense cover (Clotfelter 1998, Hauber and Russo 2000), abandoning parasitized nests (Graham 1988, Yasukawa and Werner 2007), recognizing and ejecting, puncturing, or burying parasite eggs (Graham 1988, Valera et al. 1997, Lahti 2006), or directing parental care to their own offspring instead of the foreign chick (Lichtenstein 2001, Peer et al. 2005). These host adaptations exert selective pressures on the parasites to become better at exploiting their hosts, and the parasite counter-adaptations in turn favor hosts that are even better at avoiding parasitism, producing a co-evolutionary arms race in which an adaptation in one species selects for a counter-adaptation in the other (Dawkins and Krebs 1979).

Rothstein (1970) noted that the most effective antiparasite adaptation is to avoid being parasitized in the first place, thus one strategy to reduce the cost of brood parasitism is for hosts to frontload nest defense against parasites (Welbergen and Davies 2009, Kilner and Langmore 2011, Feeney et al. 2012, Feeney and Langmore 2015). One potentially frontloaded defense is to begin incubating prior to clutch completion to limit parasite access to the nest (Uyehara and Narins 1995, Clotfelter and Yasukawa 1999). A second potentially frontloaded defense is to defend nests aggressively against parasites (Moksnes et al. 1991, Davies et al. 2003, Welbergen and Davies 2009, Medina and Langmore 2016). Although aggression against brood parasites is commonly observed (Davies 2000), we know little about its effectiveness (Welbergen and Davies 2009, Medina and Langmore 2016) or even if it is a specific

adaptation against brood parasitism rather than a general expression of antipredator behavior (Sealy et al. 1998). The frontloaded parasite-defense hypothesis predicts that aggressive nest defense is directed toward a brood parasite threatening a nest and that antiparasite aggression reduces the risk of parasitism.

The Brown-headed Cowbird (*Molothrus ater*; hereafter: cowbird) is a generalist obligate brood parasite that uses >200 host species to rear its offspring (Friedmann and Kiff 1985). To parasitize a nest successfully, a cowbird must find a suitable host nest, evade behavioral host defenses, and rapidly lay its egg (Davies 2000). In addition, a cowbird frequently removes a host egg, sometimes during a separate visit (Lowther 1993).

The Red-winged Blackbird (*Agelaius phoeniceus*; hereafter: redwing) is a commonly used host species that accepts and incubates cowbird eggs, and cares for cowbird nestlings and fledglings (Rothstein 1975b) despite the fitness cost of parasitism (Røskaft et al. 1990, Clotfelter and Yasukawa 1999, Hoover et al. 2006). Because they are so common, redwings may be the host species that produces the greatest number of cowbird fledglings across this parasite's range (Lowther 1993). Many authors have asked why redwings do not eject cowbird eggs to prevent the recoverable costs of parasitism (Rothstein 1975b, Røskaft et al. 1990, Clotfelter and Yasukawa 1999, Henger and Hauber 2014), and several hypotheses have been proposed to explain this failure to eject cowbird eggs. The evolutionary lag hypothesis proposes that redwings have not had enough time to evolve ejection behavior (Ward et al. 1996), whereas the evolutionary equilibrium hypothesis suggests that the cost of ejection is too great or the benefit too small to make ejection adaptive (Rohwer et al. 1989; Lorenzana and Sealy 1999, 2001). Alternatively, mechanical or perceptual constraints may prevent egg or chick ejection even if it were evolutionarily advantageous (Rohwer and Spaw 1988).

Redwings have a variety of antipredator behaviors (Yasukawa et al. 1992, Beletsky 1996), which could be usurped to frontload parasite defense (Henger and Hauber 2014). Alarm calling, mobbing, and physically attacking cowbird intruders, as they do nest predators, have all been observed at redwing nests during presentations of taxidermic mounts of female cowbirds (reviewed by Henger

and Hauber 2014) and may prevent a cowbird from laying its egg (Robertson and Norman 1976, 1977; Strausberger 2001; Peer et al. 2005; Hauber 2014).

We replicated the experimental methods of Henger and Hauber (2014), which they used in New York State, at a study site in Wisconsin where individual male redwings are color banded and their reproductive efforts are closely tracked. Specifically, we presented taxidermic mounts of a female and male cowbird as well as a female Northern Cardinal (*Cardinalis cardinalis*; hereafter cardinal) during laying and early incubation to test the hypothesis that redwings frontload their antiparasite defenses by aggressively preventing female cowbirds from approaching and parasitizing their nests (Feeney et al. 2012, Henger and Hauber 2014). We used a female cardinal as an experimental control because cardinals co-occur with redwings, are approximately the same size as cowbirds, and are neither brood parasites nor nest predators (Halkin and Linville 1999).

#### Comparison 1

The frontloaded parasite-defense hypothesis (FPDH) predicts that the female cowbird mount will elicit the most aggressive host responses, because it represents the greatest threat to the fitness of the host; whereas, hosts will be least aggressive toward the female cardinal mount, because it represents the lowest threat to host fitness (Henger and Hauber 2014). Responses to the male cowbird should be intermediate, because male cowbirds cannot lay parasitic eggs, but they do attract and defend females, and may assist the female in searching for host nests (Strausberger 1998). The predicted difference in response to male and female cowbirds provides a critical test, because it differs from the equally aggressive response we would expect if redwings were defending their nests against a potential predator.

#### Comparison 2

As with antipredator behavior (Knight and Temple 1986, Curio 1993, Martin 2014), aggressiveness toward a brood parasite may be affected by past experience with brood parasites or parasitism (Robertson and Norman 1976, 1977; Folkers and Lowther 1985). We compared the range and extent of aggressive behaviors exhibited

by redwings locally at nests that had been naturally parasitized by cowbirds prior to our mount presentations with those that had not been parasitized in our sample to determine whether experience with cowbird parasites influences redwing antiparasite defense. We did not have information about experience with cowbirds at previous nesting attempts, but see Hoover et al. (2006). The FPDH predicts that unparasitized nests will be more aggressively defended than parasitized nests, as more aggressive defenders should be more successful at preventing parasitism.

#### Comparison 3

The FPDH predicts that more aggressive responses should reduce parasitism rates, but the intensity of antiparasite defense is also thought to be a positive function of the population-level risk of parasitism (Rothstein 1975a, Robertson and Norman 1976) or the historical duration of parasite-host sympatry (Robertson and Norman 1977, Freeman et al. 1990, Prather et al. 1996). Robertson and Norman (1977) found a positive correlation across species between aggression toward model cowbirds and rate of parasitism, and that hosts with the longer history of sympatry with cowbirds were more aggressive than hosts that became sympatric more recently. Freeman et al. (1990) compared results of 16 studies of cowbird parasitism of redwings and found higher rates of parasitism in locations where cowbirds and redwings have a long history of parasitism than in locations where the two species have only recently come into contact, but Prather et al. (1996) did not find a significant difference in responses of redwings in locations that differed in history of sympatry.

Here, we directly compare results of cowbird presentations at our study site in Wisconsin, where parasitism is common (15% of 1,942 redwing nests were parasitized by cowbirds in 1984–2014 and 18% were parasitized in 2015) and where there is a long history of sympatry between redwings and cowbirds (Freeman et al. 1990), and the New York sites of Henger and Hauber (2014) where parasitism is rare (0% in Ithaca in 1997–2002 and 8.3% in 2010 for all sites combined) and sympatry more recent (Lowther 1993) by using the same field and statistical

methods to elicit and to quantify, respectively, redwing responses to simulated intruders.

## METHODS

### Study Species and Location

We studied the antiparasitic behavior of redwings defending active nests at Newark Road Prairie in Rock County, Wisconsin, USA (42°32'N, 89°08'W) from April to July 2015. Newark Road Prairie is a 13-ha wet-mesic remnant prairie and sedge meadow habitat that supports about 35 redwing territories (Yasukawa 1989). All male redwings were banded with United States Geological Survey numbered aluminum bands and unique color combinations of plastic wraparound bands for individual identification (USGS permit # 20438). Most females were not banded. We used the behavior of female and male redwings to locate active redwing nests and monitored their contents throughout the study. We compared results from Newark Road Prairie with those gathered at locations in New York and Ithaca, New York, USA by Henger and Hauber (2014).

### Presentation of Mounts

We replicated the methods of Henger and Hauber (2014) by using their taxidermic mounts (courtesy of Bill Strausberger) and vocalization playback files to perform presentations at redwing nests with eggs. We did not present the second, no-mount control of Henger and Hauber (2014), because of the well documented difference between no-mount and cowbird-mount presentations (reviewed in Henger and Hauber [2014] Supplementary Materials: S Table 1). All presentations occurred between 0500 and 0800 Central Daylight Time (CDT). We obtained approval for all activities from the Beloit College Institutional Animal Care and Use Committee (Protocol Number 13-001).

In each presentation, all three models (female cardinal, male cowbird, female cowbird) were used in a random, balanced order. We pushed a metal rod into the ground 1–3 m from the nest and clamped a dowel, to which the model was attached, to the rod at a height of ~1.5 m. The model was always positioned facing the nest. The playbacks comprised 10 repetitions of 10 sec of

vocalization followed by 20 sec of silence for a total of 5 min to represent a typical singing schedule. A third generation iPod Touch (Apple Inc., Cupertino, CA, USA) was connected to an Ecoxgear ECOXBT speaker (Grace Digital Audio, Peterborough, ON, Canada) via an auxiliary cable to broadcast calls at 50–55 dB SPL at 1 m from the source (Pyle PSPL01, Pyle Audio Inc., Brooklyn, NY, USA), which approximated natural call amplitude. We used one mount per species/sex, and we acknowledge this methodological limitation explicitly (Henger and Hauber 2014). Two exemplars of each species- and sex-specific vocalization were used for the male cowbird, female cowbird, and female cardinal to reduce additional pseudoreplication concerns (Henger and Hauber 2014).

Successive presentations at each nest were separated by 30–45 min to allow redwings to return to normal behavior (Honza et al. 2006). Although we presented the three mounts at 50 nests, because we used up to four nests on a single male's territory, we restricted our analysis to the first set of mount presentations to each of 25 males to avoid pseudoreplication. By limiting analysis to presentations of all three mounts at one nest per male, we also minimized the chances of using a female more than once even though most females were not banded for individual identification. Banded females were never used more than once.

### Response Variables

We used the ethogram of Henger and Hauber (2014: Supplementary Materials S Table 2) to record the same behavioral variables on check sheets using zero-one scan sampling every 15 sec for 5 min (20 total scan periods in each presentation). To estimate responses to the three mounts, we chose response variables based on the Principal Components analysis of Henger and Hauber (2014: Supplementary Materials S Tables 3–5). We tested the response variables: 1) number of presentations in which >1 male responded, 2) number of presentations in which >1 female responded, 3) number of 15-sec periods in which the male flew over the mount, 4) number of periods in which the male attacked the mount (in contrast to Henger and Hauber [2014], we combined hovers, dives, and strikes because they

were highly correlated), 5) number of periods in which the male was <3 m from the mount, 6) number of periods in which the male was perched and looking at (bill oriented towards) the mount, 7) number of periods in which the male gave ‘check’ vocalizations, 8) number of periods in which the male gave ‘whistle’ vocalizations, 9) number of times the female struck the mount, and 10) number of periods in which the female perched and looked at the mount.

Daily nest checks allowed us to determine whether each nest used in our mount presentations was naturally parasitized by cowbirds prior to our mount presentations. This information was used in a comparison of attacks of the female cowbird mount by male and female redwings at parasitized and unparasitized nests to determine whether recent experience with parasites or parasitism affected host behavior. We did not have information on previous experience with parasitism, however.

Comparisons of redwing behavior at Newark Road Prairie (Wisconsin) and New York State used the Principal Components analysis of Henger and Hauber (2014), who focused on four Principal Components interpreted as ‘Aggressive Response’, ‘Aggressive Male Vocalizations’, ‘Close Male, Female Any Distance’, and ‘Intimidation’ in response to the female cowbird mount. We used these same four components to calculate PC scores for our 25 presentations in Wisconsin and 11 presentations in New York (Henger and Hauber 2014). To ensure that any differences between locations was specific to brood parasites, we conducted the same Principal Component Analysis and calculated the same PC scores for both responses to the female cowbird mount and to the female cardinal mount.

#### Statistical Analysis

We used JMP version 11.1.1 (SAS Institute Inc. 2014) to perform all statistical analyses except Friedman’s repeated measures 2-factor analysis (via an Excel spreadsheet). All inference tests of single response variables used Wilcoxon matched-pairs tests, corrected for multiple comparisons (Šidák method), because data were not normally distributed. Tests of Principal Component scores used repeated measures ANOVA with Tukey HSD multiple comparisons because the assumption of

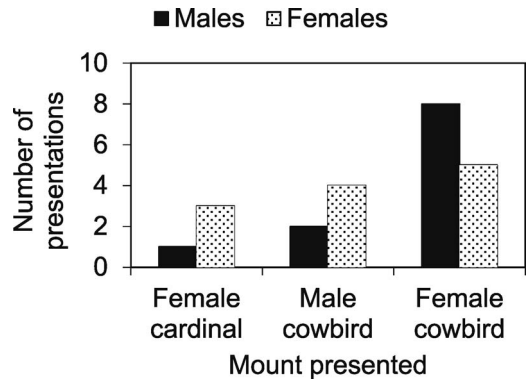


FIG. 1. Presentations in which >1 male and >1 female Red-winged Blackbird responded to three mounts ( $n = 25$  nests for all comparisons).

normality was met. Statistical significance was accepted at  $\alpha = 0.05$ .

## RESULTS

### Comparison 1

We presented the three mounts at 25 nests, each defended by a different male. Analysis of single response variables showed that the female cowbird mount elicited the most aggressive responses of redwings, and the female cardinal mount the least aggressive responses, in our presentations.

Specifically, as shown in Figure 1, the number of times more than one male redwing responded differed significantly among mounts (Log-likelihood  $G_2 = 7.86$ ,  $P = 0.020$ ), but the responses of female redwings were not significantly different between different stimuli ( $G_2 = 0.60$ ,  $P = 0.74$ ).

Figure 2 shows responses of male redwings to the three mounts. Male attacks differed significantly among mounts (Friedman’s repeated measures  $\chi_r^2 = 13.6$ ,  $n = 25$ ,  $P = 0.001$ ) and all pairwise comparisons were significant (Wilcoxon matched-pairs, multiple comparison-corrected  $\alpha$ ;  $P < 0.05$ ). The female cowbird mount was most often attacked, whereas the female cardinal mount was attacked least often. Male redwings perching within 3 m differed significantly among mounts ( $\chi_r^2 = 18.5$ ,  $n = 25$ ,  $P < 0.001$ ) and male <3 m for each of the two sexes of the cowbird mounts was significantly different than for the cardinal mount (male cowbird  $S = 57.5$ ,  $n = 25$ ,  $P < 0.05$ ; female cowbird  $S = 105.0$ ,  $n = 25$ ,  $P < 0.05$ ). Male

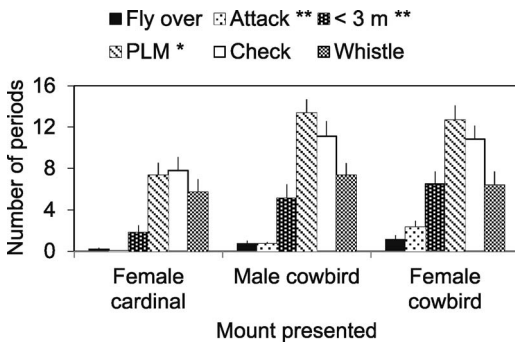


FIG. 2. Mean (+ SE) male Red-winged Blackbird responses to three mounts. <3 m = approach to within 3 m; PLM = perching and looking at the mount; check = check call; whistle = whistle call ( $n = 25$  nests for all comparisons). \*  $P < 0.05$ . \*\*  $P < 0.01$ .

perching and looking at the mount (PLM) differed significantly among mounts ( $\chi^2 = 6.00, n = 25, P = 0.049$ ) and PLM for both cowbird mounts was significantly different than for the cardinal mount (male cowbird Wilcoxon matched-pairs  $S = 227.5, n = 25, P < 0.05$ ; female cowbird  $S = 301.0, n = 25, P < 0.05$ ). Female redwing PLM and attacks did not differ among mounts (Friedman's repeated measures  $P > 0.05$ ).

Figure 3 shows redwing responses using the Principal Component scores of Henger and Hauber (2014). Scores differed significantly among mounts for Aggressive Response, Close Male, Female Any Distance, and Intimidation (repeated

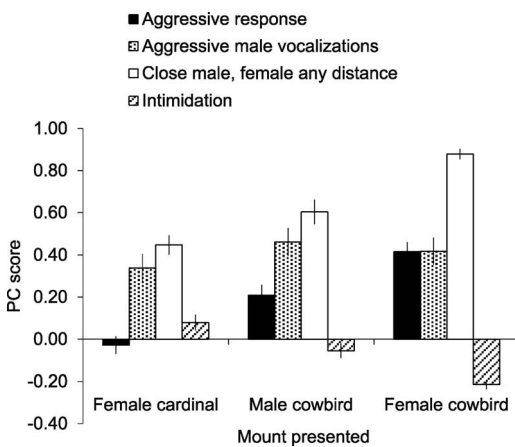


FIG. 3. Mean ( $\pm$  SE) Principal Component scores for Red-winged Blackbirds responding to three mounts. PC scores were calculated and interpreted as in Henger and Hauber (2014) ( $n = 25$  nests for all comparisons).

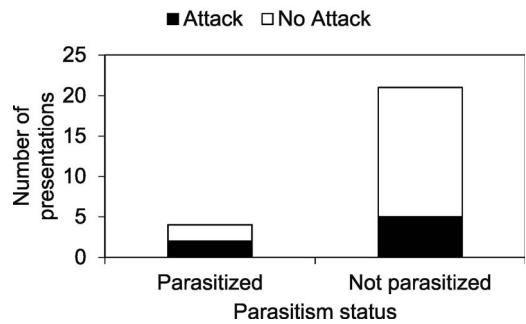
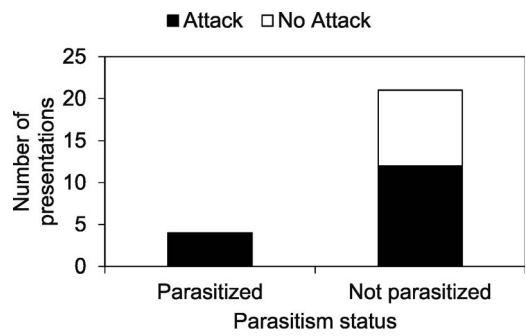


FIG. 4. Likelihood of attacking a female Brown-headed Cowbird mount versus parasitism status of Red-winged Blackbirds defending naturally parasitized nests ( $n = 4$ ) and nonparasitized nests ( $n = 21$ ). Top: males. Bottom: females.

measures ANOVA,  $P < 0.001$ ) and for all pairwise comparisons (Tukey HSD,  $P < 0.05$ ). The female cowbird mount elicited the highest Aggressive Response scores and Close Male, Female Any Distance scores, whereas the female cardinal the lowest scores. Intimidation scores were lowest (more negative) in response to the female cowbird mount. The Intimidation score depends primarily on the behaviors hover, dive, and no reaction, so that more negative scores represent when redwings are more likely to attack cowbirds than to dive or hover near them (Henger and Hauber 2014). Aggressive Male Vocalization scores did not differ significantly among mounts ( $F_{2,48} = 2.13, P = 0.13$ ).

### Comparison 2

We examined the effect of direct experience with parasites by comparing attacks of the female cowbird mount by males defending naturally parasitized and nonparasitized nests. Although the sample sizes were small, parasitized males

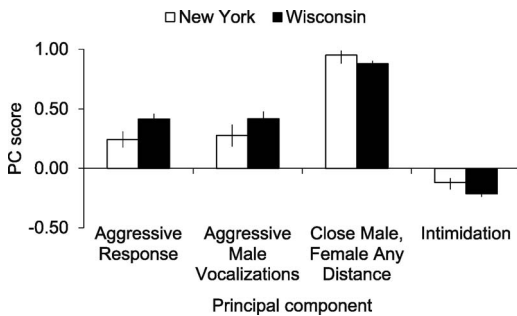


FIG. 5. Mean ( $\pm$  SE) Principal Component scores of Red-winged Blackbirds in New York State ( $n = 11$  nests) and Wisconsin ( $n = 25$  nests) responding to a female Brown-headed Cowbird mount.

were significantly more likely to attack than nonparasitized males (Log-likelihood  $G_1 = 3.99$ ,  $P = 0.046$ ).

### Comparison 3

We used the Principal Component loading coefficients of Henger and Hauber (2014) to compare responses of New York State and Wisconsin male redwings to the female cowbird mount. Figure 5 shows that males in Wisconsin responded significantly more aggressively, as measured by Principal Components Aggressive Response and Intimidation, than males in New York State ( $t_{34} = 2.16$ ,  $P = 0.038$  and  $t_{34} = -2.12$ ,  $P = 0.042$ , respectively). PC scores for Aggressive Male Vocalizations and Close Male, Female Any Distance did not differ significantly between locations, however ( $t_{34} = 1.29$ ,  $P = 0.21$  and  $t_{34} = -1.56$ ,  $P = 0.13$ , respectively). To examine the possibility that the difference in responses of redwings in Wisconsin and New York was not specific to female cowbirds, we also compared PC scores for responses to the female cardinal mount. Mean scores for three of the components, Aggressive Response, Aggressive Male Vocalizations, and Intimidation were not significantly different ( $t_{34} = -0.58$ ,  $P = 0.57$ ,  $t_{34} = 1.59$ ,  $P = 0.12$ ,  $t_{34} = 0.75$ ,  $P = 0.46$ , respectively), but we found a significant difference in Close Male, Female Any Distance ( $t_{34} = -6.18$ ,  $P < 0.001$ ). Directionally, however, the mean score for Close Male, Female Any Distance was higher in New York than Wisconsin, so there was no evidence that Wisconsin redwings were generally more responsive than New York redwings.

## DISCUSSION

### Comparison 1

Redwings responded most aggressively to the female cowbird mount, were intermediate in aggression toward the male cowbird mount, and were least aggressive toward the female cardinal mount. These results are similar to those of Henger and Hauber (2014), who found that the female cowbird mount consistently elicited the most aggressive response from redwings, and that responses to male and female cowbird mounts were different in some, but not all, Principal Component scores. Both sets of results support the frontloaded parasite defense hypothesis (FPDH).

Differences in response to model presentations of cowbirds versus nonparasites have been found in many previous studies (e.g., Robertson and Norman 1976, Ortega and Cruz 1991, Prather et al. 1999, Henger and Hauber 2014), but presentations of male and female cowbirds have produced variable results. In some cases, hosts do not differ in their responses to male and female cowbirds (e.g., Robertson and Norman 1976, Strausberger and Horning 1997, Gill et al. 2008), but other hosts may respond differently to the two sexes of the parasite (e.g., Folkers 1982, Henger and Hauber 2014). As a commonly exploited cowbird host, redwings may have evolved the ability to discriminate between and respond differently to male and female cowbirds, because they represent different levels of threat to the fitness of redwings. Unlike many other studies on redwings (reviewed by Henger and Hauber 2014), our study, identical in methods to Henger and Hauber (2014), used both visual (model) and acoustic (playback) sensory modalities to simulate parasitic (or control) intruders near the redwing nest, perhaps allowing for better discrimination between cowbird sexes and leading to more accurate assessment of the relative threat of parasitism by this host species.

### Comparison 2

We found that naturally parasitized male redwings were more aggressive toward the female cowbird mount than nonparasitized males. This result is contrary to the prediction of the FPDH. Although this result implies that aggressive nest defense is not an effective antiparasite strategy, we offer this interpretation with caution because our sample sizes were small. It is also possible that

redwings learn to become more aggressive after being parasitized. In contrast to our results, in his study at the same location in Wisconsin, Clotfelter (1998) found that parasitized and nonparasitized redwings did not differ in their aggressiveness toward a female cowbird mount and Strausberger (2001), working in northern Illinois, found that more aggressive redwings suffered lower rates of parasitism. The difference between our results and those of the prior study at our site in Wisconsin cannot be explained by a difference in parasitism rate; our rate of 18% is very similar to the 20% parasitism during Clotfelter's (1998) study. Further, we lack information on prior experience with cowbirds, either during the 2015 breeding season or during previous seasons. When comparing these three sets of results, however, it seems clear that the relationship between host antiparasite aggression and parasitism is complex. Perhaps antiparasite aggression is disadvantageous in some circumstances, but occasionally advantageous. Cowbirds were more likely to parasitize more vocally active redwings (Clotfelter 1998) and Willow Flycatchers (*Empidonax traillii*; Uyehara and Narins 1995), as well as more aggressive older Song Sparrows (*Melospiza melodia*; Smith and Arcese 1994). Conversely, the parasites may still be successfully repelled by the nest defense of aggressive hosts (Hauber 2014).

Although Freeman et al. (1990) found no differences in parasitism rates of marsh and upland redwing populations, Strausberger (2001) found that redwings nesting in marshes were rarely parasitized and always detected female cowbird mounts near their nests, whereas upland redwing colonies were more frequently parasitized and were less likely to detect female cowbird mounts. Redwings in our study in Wisconsin nested in upland habitat, whereas the nesting sites in New York were in marshes. Contrary to our results, the FPDH predicts that marsh-nesting redwings would be more aggressive towards cowbird parasites than upland redwings, because aggressive redwings are effective at preventing parasitism.

Several authors have suggested that antiparasite aggression is only effective in dense host populations (Robertson and Norman 1977, Freeman et al. 1990, Strausberger 2001) where many pairs of eyes can watch for parasites and many hosts can be recruited to mob the parasite. Model-presentation experiments showed that Superb Fairywrens

(*Malurus cyaneus*) increase their antiparasite vigilance when the risk of parasitism is high (Feeney and Langmore 2015). In low-density populations where vigilance and mobbing recruitment are limited, however, antiparasite aggression may be a cue that parasites use to find nests (Robertson and Norman 1977, Smith et al. 1984) or to assess the quality of potential hosts (Smith 1981). Further, Welbergen and Davies (2009) suggest that antipredator aggression may not be adaptive in populations with a low risk of parasitism. Although antiparasite aggression thus would be maladaptive in low-density or low-risk populations, the evolution of increased aggressiveness is favored if host fitness is increased even slightly on average by antiparasite defense (Sealy et al. 1998, see also Sih et al. 2004).

Some studies have found that antiparasite defense was associated with nest success (Clark and Robertson 1979, Folkers and Lowther 1985, Strausberger 2001), but others have found the opposite or no association (Seppä 1969; Robertson and Norman 1976, 1977; Smith 1981; Clotfelter 1998; this study). Welbergen and Davies (2009) found that antiparasite aggression of individual Reed Warblers (*Acrocephalus scirpaceus*) was highly repeatable and therefore unlikely to be influenced by previous exposure to a real parasite at their nests, but these authors also found that mobbing propensity was positively associated with parasitism risk. Given this result, we examined the repeatability of redwing antiparasite aggression using 19 males each tested at two different nests and found that male attack was significantly correlated for successive presentations at different nests of the same male (Spearman's correlation = 0.461,  $n = 19$ ,  $P = 0.047$ ). This result is consistent with a behavioral syndrome for antiparasite aggression (e.g., Sih et al. 2004, Avilés et al. 2014), and is not consistent with male redwing antipredator aggression depending on direct, past individual experience with cowbirds. Sealy et al. (1998) discussed the methods used in studies of host-parasite interactions, however, and suggested that the effectiveness of host defense can only be determined when cowbirds come to lay their eggs.

### Comparison 3

We found that redwings in Wisconsin were more aggressive toward the female cowbird mount



than redwings in New York State (also see Armstrong 2002). Our study area in Wisconsin (see Schorger 1937) lies within the traditional (long history) area of redwing-cowbird sympatry (Freeman et al. 1990), and our 32-year parasitism rate of 15% is similar to the ~22% parasitism for traditional upland habitats of Freeman et al. (1990). In contrast, parasitism is rare (in most years 0%) in the populations in New York (Henger and Hauber 2014) into which cowbirds have only recently spread (Mayfield 1965, Davies 2000). Several studies have found that hosts with a long history of sympatry with cowbirds are more aggressive toward them (reviewed in Røskaft et al. 2002), and Briskie et al. (1992) showed that hosts attacked female cowbird mounts more aggressively in sympatric than allopatric populations. Freeman et al. (1990) also found that host populations only recently sympatric with cowbirds have lower parasitism rates than host populations with a long history of sympatry, but they note also that geographic variation in the rate of parasitism may depend on relative host (especially other preferred hosts) and parasite abundance, and habitat effects on the ability of redwings to nest at high density and the ability of cowbirds to survey for nests of redwings. Once evolved, antiparasite adaptations can persist for very long periods even in allopatry (Peer et al. 2011). At this point, we suspect that the expression of antiparasite aggression reflects evolutionary, ecological, and developmental processes rather than direct experience with parasites or parasitism *per se* during the breeding history of individual hosts.

## CONCLUSIONS

Our results support some of the predictions of the hypothesis that redwings frontload their defenses to prevent cowbirds from parasitizing their nests: redwings most aggressively attack female cowbirds that represent the most immediate risk of parasitism. However, aggressive nest defense does not directly translate into lower rates of parasitism at our study site in Wisconsin, both at micro- and the macro-scale comparisons. Combating parasitism in the first place may thus be sufficient in redwings and other acceptor host species, making potential advantages of egg ejection too small to favor its evolution.

Parasite egg ejection would be favored when the benefits of ejection exceed costs such as damaging or ejecting a host's own eggs (Røskaft et al. 1990, Lorenzana and Sealy 2001). If redwings were to use puncture ejection, then the costs are potentially high (Rohwer et al. 1989), but costs of grasp ejection are considerably lower (Lorenzana and Sealy 2001). Røskaft et al. (1990) concluded that redwings could use grasp ejection, but there is some evidence that redwings in western Nebraska attempt to roll cowbird eggs out of their nests using the sides of their bills (Kren 1996). This method is often unsuccessful and because cowbird eggs are thicker shelled than redwing eggs (Spaw and Rohwer 1987), the cost of damage to host eggs as the cowbird egg rolls back may prevent this form of ejection from spreading.

We also attempted to examine the relationship between antiparasite aggression and natural parasitism, but given the high variance in results of multiple studies, evidence for a behavioral syndrome, and our small sample sizes, we cannot conclude with confidence that our results support the developmental hypothesis that experience with natural parasitism enhances the antipredator aggressiveness of host redwings. Finally, redwings in Wisconsin were more aggressive toward the female cowbird mount than redwings in New York, but because the two locations differed in habitat, parasitism rate, and history of interaction (among other things), we cannot determine whether ecological or evolutionary processes were the primary factors affecting the aggressiveness of host responses to parasites. Given the variance in results among studies, a meta-analysis of redwings' antiparasitic strategies, now including our new data, might be a productive next step of research.

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## LITERATURE CITED

- ARMSTRONG, M. A. 2002. Defenses of Red-winged Blackbirds against brood parasites and predators: the acquisition of threat recognition and the dynamics of group defense. Dissertation. State University of New York, Binghamton, USA.
- AVILÉS, J. M., E. M. BOOTELLA, M. MOLINA-MORALES, AND J. G. MARTÍNEZ. 2014. The multidimensionality of behavioural defences against brood parasites: evidence for a behavioural syndrome in magpies? *Behavioral Ecology and Sociobiology* 68:1287–1298.
- BELETSKY, L. 1996. The Red-winged Blackbird: the biology of a strongly polygynous songbird. Academic Press, San Diego, California, USA.
- BRISKIE, J. V., S. G. SEALY, AND K. A. HOBSON. 1992. Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution* 46:334–340.
- CLARK, K. L. AND R. J. ROBERTSON. 1979. Spatial and temporal multi-species nesting aggregations in birds as anti-parasite and anti-predator defenses. *Behavioral Ecology and Sociobiology* 5:359–371.
- CLOTFELTER, E. D. 1998. What cues do Brown-headed Cowbirds use to locate Red-winged Blackbird host nests? *Animal Behaviour* 55:1181–1189.
- CLOTFELTER, E. D. AND K. YASUKAWA. 1999. Impact of brood parasitism by Brown-headed Cowbirds on Red-winged Blackbird reproductive success. *Condor* 101:105–114.
- CURIO, E. 1993. Proximate and developmental aspects of antipredator behavior. *Advances in the Study of Behavior* 22:135–238.
- DAVIES, N. B. 2000. Cuckoos, cowbirds and other cheats. T. and A. D. Poyser, London, United Kingdom.
- DAVIES, N. B., S. H. M. BUTCHART, T. A. BURKE, N. CHALINE, AND I. R. K. STEWART. 2003. Reed warblers guard against cuckoos and cuckoldry. *Animal Behaviour* 65:285–295.
- DAWKINS, R. AND J. R. KREBS. 1979. Arms races between and within species. *Proceedings of the Royal Society of London, Series B* 205:489–511.
- FEENEY, W. E. AND N. E. LANGMORE. 2015. Superb Fairy-wrens (*Malurus cyaneus*) increase vigilance near their nest with the perceived risk of brood parasitism. *Auk: Ornithological Advances* 132:359–364.
- FEENEY, W. E., J. A. WELBERGEN, AND N. E. LANGMORE. 2012. The frontline of avian brood parasite–host coevolution. *Animal Behaviour* 84:3–12.
- FOLKERS, K. L. 1982. Host behavioral defenses to cowbird parasitism. *Kansas Ornithological Society Bulletin* 33:32–34.
- FOLKERS, K. L. AND P. E. LOWTHER. 1985. Responses of nesting Red-winged Blackbirds and Yellow Warblers to Brown-headed Cowbirds. *Journal of Field Ornithology* 56:175–177.
- FREEMAN, S., D. F. GORI, AND S. ROHWER. 1990. Red-winged Blackbirds and Brown-headed Cowbirds: some aspects of a host-parasite relationship. *Condor* 92:336–340.
- FRIEDMANN, H. AND L. F. KIFF. 1985. The parasitic cowbirds and their hosts. *Proceedings of the Western Foundation of Vertebrate Zoology* 2:225–304.
- GILL, S. A., D. L. H. NEUDORF, AND S. G. SEALY. 2008. Do hosts discriminate between sexually dichromatic male and female Brown-headed Cowbirds? *Ethology* 114:548–556.
- GRAHAM, D. S. 1988. Responses of five host species to cowbird parasitism. *Condor* 90:588–591.
- HALKIN, S. L. AND S. U. LINVILLE. 1999. Northern Cardinal (*Cardinalis cardinalis*). The birds of North America. Number 440.
- HAUBER, M. E. 2001. Site selection and repeatability in Brown-headed Cowbird (*Molothrus ater*) parasitism of Eastern Phoebe (*Sayornis phoebe*) nests. *Canadian Journal of Zoology* 79:1518–1523.
- HAUBER, M. E. 2014. Mafia or farmer? Coevolutionary consequences of retaliation and farming as predatory strategies upon host nests by avian brood parasites. *Coevolution: An Open Access Journal* 2:18–25. doi:10.1080/23256214.2014.913974
- HAUBER, M. E. AND S. A. RUSSO. 2000. Perch proximity correlates with higher rates of cowbird parasitism of ground nesting Song Sparrows. *Wilson Bulletin* 112:150–153.
- HENGER, C. S. AND M. E. HAUBER. 2014. Variation in antiparasitic behaviors of Red-winged Blackbirds in response to simulated Brown-headed Cowbirds. *Wilson Journal of Ornithology* 126:488–499.
- HONZA, M., V. ŠIČHA, P. PROCHÁZKA, AND R. LEŽALOVÁ. 2006. Host nest defense against a color-dimorphic brood parasite: Great Reed Warblers (*Acrocephalus arundinaceus*) versus Common Cuckoos (*Cuculus canorus*). *Journal of Ornithology* 147:629–637.
- HOOVER, J. P. AND S. K. ROBINSON. 2007. Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. *Proceedings of the National Academy of Sciences of the USA* 104:4479–4483.
- HOOVER, J. P., K. YASUKAWA, AND M. E. HAUBER. 2006. Spatially and temporally structured avian brood parasitism affects the fitness benefits of hosts' rejection strategies. *Animal Behaviour* 72:881–890.
- KILNER, R. M. AND N. E. LANGMORE. 2011. Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biological Reviews* 86:836–852.
- KNIGHT, R. L. AND S. A. TEMPLE. 1986. Why does intensity of avian nest defense increase during the nesting cycle? *Auk* 103:318–327.
- KREN, J. 1996. Proximate and ultimate mechanisms of Red-winged Blackbird (*Agelaius phoeniceus*) responses to interspecific brood parasitism. Dissertation. University of Nebraska, Lincoln, USA.
- LAHTI, D. C. 2006. Persistence of egg recognition in the absence of cuckoo brood parasitism: pattern and mechanism. *Evolution* 60:157–168.
- LICHTENSTEIN, G. 2001. Low success of Shiny Cowbird chicks parasitizing Rufous-bellied Thrushes: chick–chick competition or parental discrimination? *Animal Behaviour* 61:401–413.
- LORENZANA, J. C. AND S. G. SEALY. 1999. A meta-analysis of the impact of parasitism by the Brown-headed Cowbird on its hosts. *Studies in Avian Biology* 18:241–253.

- LORENZANA, J. C. AND S. G. SEALY. 2001. Fitness costs and benefits of cowbird egg ejection by Gray Catbirds. *Behavioral Ecology* 12:325–329.
- LOWTHER, P. E. 1993. Brown-headed Cowbird (*Molothrus ater*). The birds of North America. Number 47.
- MARTIN, C. W. 2014. Naïve prey exhibit reduced antipredator behavior and survivorship. *PeerJ* 2:e665.
- MAYFIELD, H. 1965. The Brown-headed Cowbird, with old and new hosts. *Living Bird* 4:13–28.
- MEDINA, I. AND N. E. LANGMORE. 2016. Batten down the hatches: front-line defences in an apparently defenceless cuckoo host. *Animal Behaviour* 112:195–201.
- MOKSNES, A., E. RØSKAFT, A. T. BRAA, L. KORSNES, H. M. LAMPE, AND H. C. PEDERSEN. 1991. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* 116:64–89.
- MOSKÁT, C., Z. BARTA, M. E. HAUBER, AND M. HONZA. 2006. High synchrony of egg laying in Common Cuckoos (*Cuculus canorus*) and their Great Reed Warbler (*Acrocephalus arundinaceus*) hosts. *Ethology Ecology and Evolution* 18:159–167.
- ORTEGA, C. P. AND A. CRUZ. 1991. A comparative study of cowbird parasitism in Yellow-headed Blackbirds and Red-winged Blackbirds. *Auk* 108:16–24.
- PEER, B. D., M. J. KUEHN, S. I. ROTHSTEIN, AND R. C. FLEISCHER. 2011. Persistence of host defence behaviour in the absence of avian brood parasitism. *Biology Letters* 7:670–673.
- PEER, B. D., S. I. ROTHSTEIN, M. J. KUEHN, AND R. C. FLEISCHER. 2005. Host defenses against cowbird (*Molothrus* spp.) parasitism: implications for cowbird management. *Ornithological Monographs* 57:84–97.
- PRATHER, J. W., C. P. ORTEGA, AND A. CRUZ. 1999. Aggressive responses of Red-winged Blackbirds (*Agelaius phoeniceus*) toward Brown-headed Cowbirds (*Molothrus ater*) in areas of recent and long-term sympatry. *Bird Behavior* 13:1–7.
- ROBERTSON, R. J. AND R. F. NORMAN. 1976. Behavioral defenses to brood parasitism by potential hosts of the Brown-headed Cowbird. *Condor* 78:166–173.
- ROBERTSON, R. J. AND R. F. NORMAN. 1977. The function and evolution of aggressive host behavior towards the Brown-headed Cowbird (*Molothrus ater*). *Canadian Journal of Zoology* 55:508–518.
- ROHWER, S. AND C. D. SPAW. 1988. Evolutionary lag versus bill-size constraints: a comparative study of the acceptance of cowbird eggs by old hosts. *Evolutionary Ecology* 2:27–36.
- ROHWER, S., C. D. SPAW, AND E. RØSKAFT. 1989. Costs to Northern Orioles of puncture-ejecting parasitic cowbird eggs from their nests. *Auk* 106:734–738.
- RØSKAFT, E., G. H. ORIANS, AND L. D. BELETSKY. 1990. Why do Red-winged Blackbirds accept eggs of Brown-headed Cowbirds? *Evolutionary Ecology* 4:35–42.
- RØSKAFT, E., A. MOKSNES, B. G. STOKKE, V. BICÍK, AND C. MOSKÁT. 2002. Aggression to dummy cuckoos by potential European Cuckoo hosts. *Behaviour* 139:613–628.
- ROTHSTEIN, S. I. 1970. An experimental investigation of the defenses of the hosts of the parasitic Brown-headed Cowbird (*Molothrus ater*). Dissertation. Yale University, New Haven, Connecticut, USA.
- ROTHSTEIN, S. I. 1975a. Evolutionary rates and host defenses against avian brood parasitism. *American Naturalist* 109:161–176.
- ROTHSTEIN, S. I. 1975b. An experimental and teleonomic investigation of avian brood parasitism. *Condor* 77:250–271.
- ROTHSTEIN, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics* 21:481–508.
- SAS INSTITUTE INC. 2014. JMP Pro. Version 11.1.1. SAS Institute Inc., Cary, North Carolina, USA.
- SCHORGER, A. W. 1937. The range of the bison in Wisconsin. *Transactions of the Wisconsin Academy of Sciences, Arts and Letters* 30:117–130.
- SEALY, S. G., D. L. NEUDORF, K. A. HOBSON, AND S. A. GILL. 1998. Nest defense by potential hosts of the Brown-headed Cowbird: methodological approaches, benefits of defense, and coevolution. Pages 194–211 in *Parasitic birds and their hosts: studies in coevolution* (S. I. Rothstein and S. K. Robinson, Editors) Oxford University Press, New York, USA.
- SEPPÄ, J. 1969. The cuckoo's ability to find a nest where it can lay an egg. *Ornis Fennica* 46:78–79.
- SHI, A., A. BELL, AND J. C. JOHNSON. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution* 19:372–378.
- SMITH, J. N. M. 1981. Cowbird parasitism, host fitness, and age of the host female in an island Song Sparrow population. *Condor* 83:152–161.
- SMITH, J. N. M. AND P. ARCESE. 1994. Brown-headed Cowbirds and an island population of Song Sparrows: a 16-year study. *Condor* 96:916–934.
- SMITH, J. N. M., P. ARCESE, AND I. G. McLEAN. 1984. Age, experience, and enemy recognition by wild Song Sparrows. *Behavioral Ecology and Sociobiology* 14:101–106.
- SPAW, C. D. AND S. ROHWER. 1987. A comparative study of eggshell thickness in cowbirds and other passerines. *Condor* 89:307–318.
- STRAUSBERGER, B. M. 1998. Evident nest-searching behavior of female Brown-headed Cowbirds while attended by males. *Wilson Bulletin* 110:133–136.
- STRAUSBERGER, B. M. 2001. The relationship of habitat and spatial distribution of nests with Brown-headed Cowbird parasitism of Red-winged Blackbirds. *Wilson Bulletin* 113:129–133.
- STRAUSBERGER, B. M. AND M. E. HORNING. 1997. Responses of nesting Song Sparrows (*Melospiza melodia*) and Red-winged Blackbirds (*Agelaius phoeniceus*) to models of parasitic cowbirds and nonthreatening towhees. *Bird Behavior* 12:71–78.
- UYEHARA, J. C. AND P. M. NARINS. 1995. Nest defense by Willow Flycatchers to brood-parasitic intruders. *Condor* 97:361–368.
- VALERA, F., H. HOI, AND B. SCHLEICHER. 1997. Egg burial in Penduline Tits, *Remiz pendulinus*: its role in mate desertion and female polyandry. *Behavioral Ecology* 8:20–27.

- WARD, D., A. K. LINDHOLM, AND J. N. M. SMITH. 1996. Multiple parasitism of the Red-winged Blackbird: further experimental evidence of evolutionary lag in a common host of the Brown-headed Cowbird. *Auk* 113:408–413.
- WELBERGEN, J. A. AND N. B. DAVIES. 2009. Strategic variation in mobbing as a front line of defense against brood parasitism. *Current Biology* 19:235–240.
- YASUKAWA, K. 1989. The costs and benefits of a vocal signal: the nest-associated 'chit' of the female Red-winged Blackbird, *Agelaius phoeniceus*. *Animal Behaviour* 38:866–874.
- YASUKAWA, K. AND W. WERNER. 2007. Nest abandonment as a potential anti-parasite adaptation in the Red-winged Blackbird. *Passenger Pigeon* 69:481–489.
- YASUKAWA, K., L. K. WHITTENBERGER, AND T. A. NIELSEN. 1992. Anti-predator vigilance in the Red-winged Blackbird, *Agelaius phoeniceus*: do males act as sentinels? *Animal Behaviour* 43:961–969.