VARIATION IN ANTIPARASITIC BEHAVIORS OF RED-WINGED BLACKBIRDS IN RESPONSE TO SIMULATED BROWN-HEADED COWBIRDS

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ABSTRACT.—Red-winged Blackbirds (*Agelaius phoeniceus*) are a frequently parasitized host species of obligate brood parasitic Brown-headed Cowbirds (*Molothrus ater*). Yet, this common host does not reject parasite eggs or young. A hypothesis to explain the lack of cowbird egg and nestling rejection is that redwings frontload their responses by investing into antiparasitic nest defense behaviors toward female cowbirds, relative to non-laying male cowbirds and non-threatening other species. A review of prior studies using cowbird-mount presentations with or without acoustic playbacks supported some but not all predictions of this hypothesis. We conducted a new study at two geographically separate sites, in New York City, NY and in Ithaca, NY, USA, where Red-winged Blackbirds were presented with taxidermic models on a tripod and species- and sex-specific vocalization playbacks of female or male Brown-headed Cowbirds, female Northern Cardinals (*Cardinalis cardinalis*), or a tripod and silence (control). Reactions of both sexes to mount and playback presentations near known, active nests, conducted repeatedly throughout the breeding cycle, were supplemented with mount and playback presentations to males without known nests. Red-winged Blackbirds responded more aggressively toward presentations of both sexes of the parasite than to the cardinal and the tripod treatments. In addition, they responded more aggressively at known nests to mounts presented closer rather than farther, and at nests with nestlings rather than eggs. Furthermore, aggressive reactions were more frequent in Ithaca, NY than in New York City, NY. Finally, the reactions of female and male redwings near known active nests and of males without known nests showed statistically similar patterns, implying that data from these two types of experimental contexts could be combined in our analyses, and perhaps in future studies, to more efficiently characterize this host's responses to parasite presentations. *Received 16 November 2013. Accepted 1 March 2014.*

Key words: aggression, brood parasitism, mount presentation, nest defense, sex differences.

Avian obligate brood parasites lay their eggs into other species' nests, and depend on the hosts to provision and protect their offspring (Davies 2000). Parasitism, by definition, negatively affects the fitness of the host species through the initial exchange of host eggs for parasitic eggs, decreased hatching success of host eggs, and the competition for resources between unrelated chicks (Rothstein 1990, Hauber 2003a).

Hosts employ many strategies to prevent brood parasitism. Building nests in cavities with small entrances (Hoover and Robinson 2007), in enclosed spaces (Hauber 2001) or in dense cover reduces the risk of being located by the parasites (Clotfelter 1998, Hauber and Russo 2000). Abandoning parasitized nests (Hosoi and Rothstein 2000), burying parasitic eggs (Valera et al. 1997), and ejecting parasitic eggs from the nest (Lahti 2006) are also potential means of foregoing continued care for the unrelated parasitic eggs and young. Nest desertion releases the hosts from extended provisioning for the parasites (Langmore et al. 2003, Grim 2007, Hauber et al. 2013), while it also avoids the cognitive costs and errors of discriminating own and foreign eggs (Lotem 1993, Moskat and Hauber 2007, Moskat et al. 2014).

The parasitism frequency of Red-winged Blackbirds *Agelaius phoeniceus* (hereafter: redwing) by Brown-headed Cowbirds *Molothrus ater* (hereafter: cowbird) is exceedingly variable among different geographic locations and within populations across North America, and even between different years (Ortega and Cruz 1988, Freeman et al. 1990, Clotfelter and Yasukawa 1999). Parasitism of redwings by cowbirds also varies at the microhabitat scale with vegetation characteristics, including decreasing parasitism rates with more distance from potential cowbird perch sites (Clotfelter 1998), and lower parasitism rates of marsh-nesting, densely colonial versus upland-nesting, more loosely colonial redwings (Peer et al. 2005); the latter pattern is thought to be because of more successful mobbing and nest defense by redwings against cowbirds within the more densely populated colonies (Strausberger 2001).

Despite this variation in the local likelihood of cowbird parasitism, redwings are so numerous

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throughout North America that they are considered the host species responsible for producing the most cowbird fledglings (Lowther 1993). Surprisingly, however, redwings do not prevent the many costs of cowbird parasitism through the rejection of cowbird eggs or nestlings (Rothstein 1975, Roskaft et al. 1990, Clotfelter and Yasukawa 1999). This is unexpected because redwings are indeed able to remove egg-sized objects experimentally placed in their nests, but the birds evidently do not respond to real or experimental cowbird eggs by ejecting them (Ortega et al. 1993). Moreover, cowbirds do not appear to use egg mimicry or crypsis to avoid the detection of the parasitic egg (Ortega et al. 1993). It is possible that redwings have not yet evolved the necessary adaptations to eject eggs because of evolutionary lag (Ward et al. 1996) or it may be too costly for redwings to evolve an ejection behavior because of the potential cost of damaging one or more of their own eggs while attempting to reject the relatively thicker-shelled cowbird egg (Rowher et al. 1989). Redwings do sometimes desert parasitized nests (Clotfelter and Yasukawa 1999), but it can be costly in terms of time lost building a new nest (Clotfelter and Yasukawa 1999), and there is also a greater chance of predation later in the season (Capper et al. 2012).

The redwings' primary cost of parasitism is the decrease in clutch size as the cowbird removes a redwing egg and lays its own into the nest (Hoover et al. 2006); in turn redwings suffer minimal additional costs to raise a cowbird chick in the nest alongside their own young (Lorenzana and Sealy 1999, Hauber 2003a). A hypothesized strategy to reduce the initial and non-recoverable cost of cowbird parasitism is for the redwings to frontload their defenses (Welbergen and Davies 2009, Kilner and Langmore 2011, Feeney et al. 2012) to prevent the cowbird from approaching the nest, removing a redwing egg, and laying its own egg in the nest. For example, in a different host-parasite system, Welbergen and Davies (2009) found that Eurasian Reed Warblers' (*Acrocephalus scirpaceus*) aggression toward parasitic Common Cuckoos (*Cuculus canorus*) reduced rates of parasitism.

Observations at nests and experiments with mount-presentations (reviewed in Supplementary Materials: S Table 1) have revealed that redwings vigorously defend their nests from nearby cowbirds, by alarm calling, mobbing, and physically attacking the parasitic intruders, relative to harmless heterospecific birds (Robertson and Norman 1976, Ortega and Cruz 1991, Prather et al. 1999). Critically, the responses of redwings to predatory birds vs. cowbirds are also quantitatively different (Neudorf and Sealy 1992; S Table 1), implying that even though cowbirds do occasionally prey on host eggs and nestlings, parasitic cowbirds are considered a different class of threat compared to other avian nest predators (Yasukawa et al. 1992; S Table 1). The specific hypothesis, therefore, for the evolution of the specific patterns and timing of antiparasitic defenses (and the lack thereof) by redwings against cowbirds is that this host species frontloads its defenses by predominately reducing the cost of parasitism through the prevention of parasitic egg laying in the first place rather than by recognizing and rejecting parasitic eggs or young (Røskaft et al. 1990, Grim 2011, Feeney et al. 2012).

Our study used experimental mount-presentations to examine aggressive nest-defense behaviors employed by redwings toward cowbirds. The specific aim of the study was to determine whether patterns of host behaviors represent specific antiparasitic behaviors that may function so as to prevent female cowbirds from approaching and parasitizing redwing nests. Our three sets of comparisons are described in the following paragraphs.

Comparison 1.—There has been little published research to date reporting on the rate and variance of cowbird parasitism and the potential antiparasitic responses of redwings in these species' northeastern range of sympatry, including New York State, USA (S Table 1). Previous research in Ithaca, NY (Armstrong 2002) found cowbird parasitism of redwings to be <1%, and during 5 years of research with 20+ redwing nests monitored per year within 50 km of Ithaca, NY, Hauber (2003b) found no cowbird eggs in redwing nests in either marsh or upland sites. Examining the specificity of redwings' nest defenses against parasitic cowbirds in such populations may be informative for future comparisons and meta-analyses as to the cognitive basis and ecological efficiency of antiparasitic strategies in local populations. The present study therefore explored the parasite/host relationship between redwings and cowbirds in New York State by examining nest defense behaviors in response to presentations of taxidermic models accompanied by playbacks of the respective

vocalizations of female and male cowbirds, and female Northern Cardinals (*Cardinalis cardinalis*), as a non-parasitic, non-predatory sympatric stimulus species. We hypothesized that the redwings would engage in significantly more aggressive behaviors toward the female cowbird mount than the other bird models, implying that parasitically-laying female cowbirds are considered a unique class of threat to the nesting attempt of redwings.

Comparison 2.—We compared the redwings' reactions to the mounts between New York City, NY and Ithaca, NY. This was done initially in order to increase the sample size of redwings for our study but it also allowed us to compare the consistency and variation of host behaviors between these two relatively distant sites of ~270 km apart (Rand McNally 2004), with a different ecological context (urban vs. rural setting), within the general region of northeastern North America. Our study is the first that we are aware of regarding the context of obligate brood parasitism of redwings within the urban draw of New York City (hereafter NYC).

Comparison 3.—Finally, we aimed to determine whether it was possible to study redwings' antiparasitic behavior from the behaviors of individuals without having to locate active nests in the vicinity of the parasitic mount's presentation. Locating redwing, and other cowbird-host, nests is potentially time consuming and can be logistically difficult or detrimental for the outcome of the nesting attempt, especially in the urban setting of highly controlled public parks of New York City with many potential nearby observers. If urban researchers were able to feasibly combine the data from redwing territories without known nests with data taken from responses at known active nests, it would produce a larger data set and a potentially more economical and/or robust account of local antiparasitic behaviors.

To complement the already large literature on using mount-presentations in the study of avian host-parasite interactions (reviewed in Grim 2005) and in cowbird-redwing interactions (as cited above, Strausberger and Horning 1998; S Table 1), our study was designed to provide novel experimental data in that we studied parasitism in urban areas within NYC, we conducted playbacks of sex- and species-specific vocalizations associated with each mount of parasite sex and control species, and we compared antiparasitic behaviors

from redwings defending nests to those without known nests. Moreover, our study is one of the few to use principal components to describe patterns of antiparasitic behaviors in an avian host species.

METHODS

Study Site and Species.—We located adult redwings at various parks in New York City and Ithaca, NY, USA, during the spring and summer of 2010. The search was focused on the typical redwing breeding habitats of fresh and saltwater marshes, as well as upland meadows (Searcy and Yasukawa 1995). Both redwings and cowbirds lay eggs from April to July (Lowther 1993, Searcy and Yasukawa 1995). The female Northern Cardinal (hereafter: cardinal) was used as a control bird species because cardinals are sympatric with redwings and they are approximately the same size as cowbirds, but they are neither parasitic nor do they prey upon redwing nests (Halkin and Linville 1999).

Redwings were not banded or otherwise individually identifiable as part of this study. To reduce pseudoreplication, we walked at least 50 m between presumed redwing territories (Searcy and Yasukawa 1995). In addition, each mount/control (see section below on *Presentation of Mounts*) was presented only once at each territory with a known active nest and whereas only 1 of the 4 mount/control treatments were presented at sites with no known nests. We defined a nest as active if there was at least one egg in the nest and if we observed both a male and female redwing within 5 m of the nest. At sites with no known nests, the mounts were presented only when at least one redwing male was observed perched in submerged vegetation at a likely nesting territory.

In and around Ithaca, NY, a total of three nesting pairs of redwings and 16 redwing males without known nests were exposed to the mount presentations. The presentations occurred in and around the Cornell University's Experimental Aquatic Ponds Units 1 and 2, located in Lansing, NY, USA (42° 50′ 33″ N, 76° 46′ 58″ W). The Cornell Pond Units are described in detail by Westneat (1992) and Hauber (2000). All of the sites in Ithaca were situated in rural areas, and sites behind fences, not open to the general public.

In NYC, we conducted experiments in Queens, NY, where a total of nine active nests of redwings and 18 presumed territories without known nests were exposed to the mount presentations. The

study localities in Queens included Flushing Meadows Corona Park (40.73° N, 73.84° W), Conselyea's Pond at Brookfield Park (40.65° N, 73.74° W), Baisley Pond (40.67° N, 73.78° W) and Kissena Park (40.74° N, 73.80° W). We also conducted experiments in the Bronx, NY, and the experiments included three redwing nests and nine territories without known nests. The sites in and around the Bronx, NY were Van Cortland Park (40.88° N, 73.89° W), Scout Park in Bronxville, NY (40.93° N, 73.83° W), Soundview Park (40.76° N, 73.86° W) and Shore Road (40.86° N, 73.80° W). Finally, we studied redwings in Brooklyn, NY, where the experiments included a total of eight territories without known nests. The sites in Brooklyn, NY were Prospect Park Lake (40.65° N, 73.96° W) and Marine Park (40.60° N, 73.93° W). All of the presentations done in NYC took place in public parks.

Presentation of Mounts.—We used taxidermic avian models for the experiment (courtesy of B. Strausberger Field Museum, Chicago, USA). Logistical limitations meant that only one exemplar model was used per stimulus type. Studies that have used replicate specimens for antiparasitic mount presentation experiments have found no difference between replicate dummy types (Davies and Welbergen 2008, Grim 2011). A photographic tripod served as a base upon which to mount and present each model. The tripod (height 0.8 m) was painted brown in order to provide some camouflage. The pan head was removed from the top of the tripod and in its place was attached a square piece of Styrofoam, also painted brown. Each model bird was attached to a metal rod that would fit into the Styrofoam, which allowed the models to be positioned upright. The empty tripod itself was also used as a treatment control to test whether the redwings would show aggression to a foreign object in or near their nest and territories.

Many birds recognize and respond differently to heterospecific vocalizations (Hauber et al. 2002, Forsman and Martin 2009), and so species- and sex-specific playbacks were used simultaneously with the different mounts' presentations to elicit responses from the redwings. Two exemplars of species- and sex-specific vocalizations were used each for the male cowbird, the female cowbird, and the cardinal mount, to reduce pseudoreplication concerns (Hauber et al. 2001). The vocalizations were obtained from The Digital Resource Commons of Ohio University (drc. ohiolink.edu) and www.findsounds.com. Raven

Pro 1.3 Sound Editing Software was used to generate 5-min sound files for each of the vocalizations. Each sound file consisted of 10 sec of sound followed by 20 sec of silence. The 10 sec of sound were comprised of 1 sec of vocalization followed by 2 sec of silence, repeated for 10 sec. To generate ''silent'' playbacks, the intensity of the sound was decreased to 10^{-6} of its original intensity. Thus, the control included the tripod presentation, accompanied by 5 min and one of two exemplars of ''silent'' playback.

The combined 10 sec of vocalization followed by 20 sec of silence was duplicated 10 times to generate a 5-min sound file. One min of silence (sound intensity 10^{-5} of original sound) was added to the beginning and end of each sound file, allowing time for the experimenter to position the mount at the nest at the beginning of the trial and to retrieve the mount after the trial was over without exposing the birds to a different vocalization. A final band pass filter between 500–10,000 Hz was applied for all sound files. All playbacks were standardized to a uniform peak amplitude and were played back at the maximal volume setting. Playbacks were played from an iPod Nano device connected to two portable Radioshack™ Model #40-1434 Portable Folding Speakers.

The study sites were chosen by looking for suitable redwing nesting microhabitats in parks with bodies of water surrounded by vegetation (Searcy and Yasukawa 1995). Once a suitable locality was found, we searched for male redwings since they are easier to locate than females with their larger bodies and deep black coloration, and they find and defend territories (Searcy and Yasukawa 1995). After male redwings were seen or heard, we approached them until we were no more than 2 m from the birds. At this distance, males defending nests will begin to produce alarm vocalizations or hover towards humans who approach the nest (Searcy and Yasukawa 1995). If the male redwings directed aggressive behaviors toward us, we would look in the immediate area for nests. Redwing females generally build their nests ∼1 m from the ground in marsh vegetation (Beletsky 1996), and male redwings will also intensify their aggressive behavior as humans approach closer to the nest (Searcy and Yasukawa 1995), which sometimes aids in locating the nest. If a nest was not found, only one of the mounts or the tripod, chosen in a random balanced manner, was presented to each

male redwing, regardless of whether the male was solitary or there were females present. All 4 mounts/tripod were not presented to the males without known nests, because it was not possible to know whether the same birds would be present at the site during subsequent presentation.

When a nest was found, the numbers of redwing and cowbird eggs and/or nestlings were recorded for the nest. We would then retreat to a distance at least 10 m away from the nest and wait for the redwings to stop alarm calling. During that time, the mount apparatus would be set up in preparation to present the taxidermic models. Once the birds were silent, one of the mounts or the tripod was presented to the birds. In order to keep the bird model hidden until it was presented to the birds a brown cloth was draped on top of the model.

The mount apparatus was presented as close as possible to the nest. The distance from the mount apparatus to the nest varied between 1–4 m, because the nests were usually built in dense vegetation. The mount apparatus was placed at the nearest edge of the vegetation. It was presented at the same height, 0.8 m from the ground, at each presentation. The taxidermic bird mount was always displayed facing the nest. The brown cloth covering the model was removed as soon as the apparatus was in position. The iPod and speakers were placed inside the brown cloth to camouflage them. They were then positioned on the ground just beside the mount apparatus.

At each presentation site, the apparatus was positioned at the same location during all four presentations. We started recording the behaviors for each trial as soon as the vocalizations began to play. Since the playback of the silent track did not have audible components with which we could synchronize recording, the behaviors for each control trial were recorded as soon as we were positioned 10 m away from the model. Each recording was played at the maximum volume. The birds' behaviors were recorded for 5 min, even if the birds flew away. One-zero sampling scans were used and the behaviors were recorded every 15 sec. This type of sampling was chosen in order to record rapid behaviors, such as vocalizations and strikes, which are difficult to capture with other sampling methods (Martin and Bateson 2009). Mount presentations were performed between 30–45 min apart to allow the redwings to return to their normal behavior (Honza et al. 2006). The order of presentation of the mounts

was determined in a random balanced manner. Most of the trials were videotaped using a handheld video camera. Some trials were reviewed during data analysis to determine which sex of blackbird performed a certain behavior.

Data Collection and Analysis.—We used an electronic behavioral data logger for this study by utilizing a recently developed iPhone/iPad touch application named ''WhatISee.'' (T. Heuser 2009; hereafter: App or application; described in detail by Henger et al. 2012). We generated an ethogram template for redwings by using the vocalizations and behaviors recorded in previous experiments of redwing nest defense that were found in the literature (Supplementary Materials: S Table 2).

A Principal Components Analysis was used in order to reduce the high number of possibly correlated behavioral responses into a smaller number of independent variables (Kachigan 1991, Grim 2005, see also Honza et al. 2006). The data from the Nest and No Known Nest Sites were first combined and then Principal Components (PC) scores were generated separately from behavioral, vocal, and spatial distribution data types. The Principal Components were divided into these three groups in order to characterize information about the physical and vocal reactions to the models as well as the proximity of the redwings to the models and the number of redwings that responded. Only Principal Components with Eigenvalues >1.0 were used in the statistical tests (Kachigan 1991). The Eigenvectors are shown in the Supplementary Material (S Tables 3–5).

All statistical tests were conducted in JMP 8.0 Software. A backward elimination protocol was used in a General Linear Mixed Model (GLMM) (O'Hara 2009), with all of the Principal Components as dependent variables and Nest ID as the random effect. The Principal Components are Aggressive Response, Aggressive Male Vocalizations, Aggressive Female Vocalizations, Female Strikes, Intimidation, Close Multiple Males and Females, Close Male, Distant Female, Close Male, Female Any Distance, and Other Birds. The experimentally controlled predictor variables were Location, Type of Mount, Mount Presentation Order, and Nest Present or Absent; correlative predictors included Eggs or Nestlings, and Distance from Model.

All of the predictors were added to the model and non-significant predictors were removed one at a time. We stopped removing predictors when all of the remainders in the model showed a
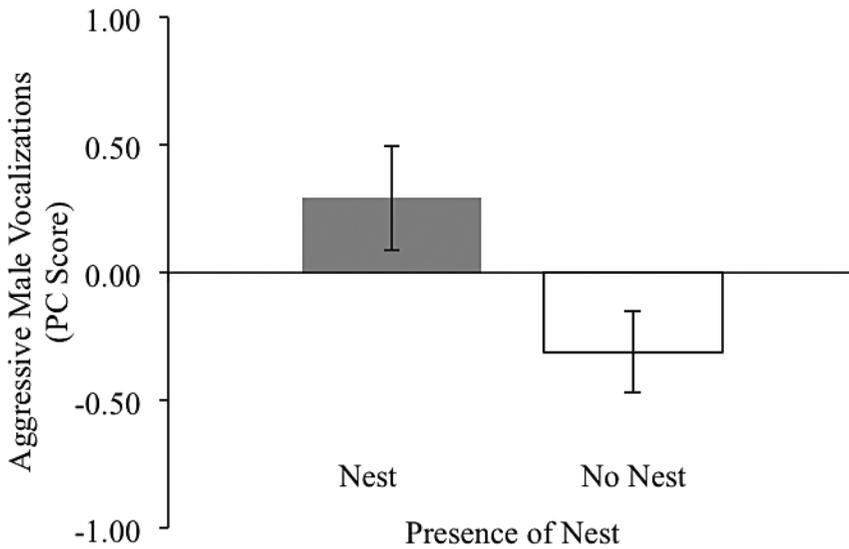
FIG. 1. Differences in the responses (least square mean ± SE) of Red-winged Blackbirds across all treatments (PC score: ''Aggressive Male Vocalizations'') depending on whether there was a known active nest in the vicinity of the mount-playback presentation experiment. This difference was significant at $P < 0.05$.

significant effect at $\alpha = 0.05$. The GLMM approach provides a numerical approximation of degrees of freedom through the Kenward-Roger method, so the reported values are not necessarily whole numbers (Kenward and Roger 1997). There were no a priori predictions for interactions. Two Principal Components (''Close Multiple Males and Females'' and ''Other Bird Species'') did not meet the assumptions of parametric tests and so they were $\log_{10} (x + 10)$ transformed prior to analyses.

RESULTS

Comparison 1.—For four of the PC scores, there were significant differences between the redwings' reactions to the different types of stimulus presentations. Considering the PC score ''Aggressive Response,'' redwings showed significantly higher PC scores in reaction to both the female cowbird and male cowbird than the cardinal or the tripod (pooled data: $r^2 = 0.48$, $F_{3,82.46} = 5.98$, $P = 0.001$, Fig. 2a). Significantly higher values of the PC score ''Aggressive Male Vocalizations'' were recorded when redwings were reacting to the male and female cowbirds compared to the cardinal or the tripod (No Known Nest data only: $r^2 = 0.24$, $F_{3,15.39} = 4.98$, $P = 0.004$, Fig. 2b). Responses toward the female parasite produced significantly higher PC scores than responses to the male parasite and the tripod

for the PC score ''Close Male, Female Any Distance'' (pooled data: $r^2 = 0.76$, $F_{3,53.8} = 3.22$, $P = 0.030$, Fig. 2c). Responses toward the female cowbird produced significantly lower values than responses toward the three other models for the PC score ''Intimidation'' (pooled data: $r^2 = 0.83$, $F_{3,49.22} = 5.13$, $P = 0.004$, Fig. 2d).

There was no significant difference between reactions to the stimulus presentations for the following PCs when all data were combined: ''Female Strike'' ($r^2 = 0.11$, $F_{3,91.74} = 1.34$, $P = 0.27$), ''Aggressive Female Vocalizations,'' ($r^2 = 0.47$, $F_{3,75.74} = 1.10$, $P = 0.35$) ''Close Multiple Males & Females'' ($r^2 = 0.43$, $F_{3,76.66} = 2.40$, $P = 0.076$) ''Close Male, Distant Females'' ($r^2 = -0.36$, $F_{3,92.61} = 2.17$, $P = 0.097$), and ''Other Bird Species'' ($r^2 = 0.14$, $F_{3,80.67} = 1.17$, $P = 0.33$).

Comparison 2.—The PC scores ''Male Aggressive Vocalizations'' were higher in Ithaca than in NYC (Known Nest data only: $r^2 = 0.70$, $F_{1,9.56} = 17.243$, $P = 0.002$, Fig. 3a).

Comparison 3.—The PC score ''Aggressive Male Vocalizations'' was the only PCA metric that was significantly different depending on whether redwings were presented with the mounted model(s) and playback(s) with or without a known active nest; the values were higher near known nests ($r^2 = 0.74$, $F_{1,42.45} = 5.80$, $P = 0.02$, Fig. 1); all other PC scores were statistically
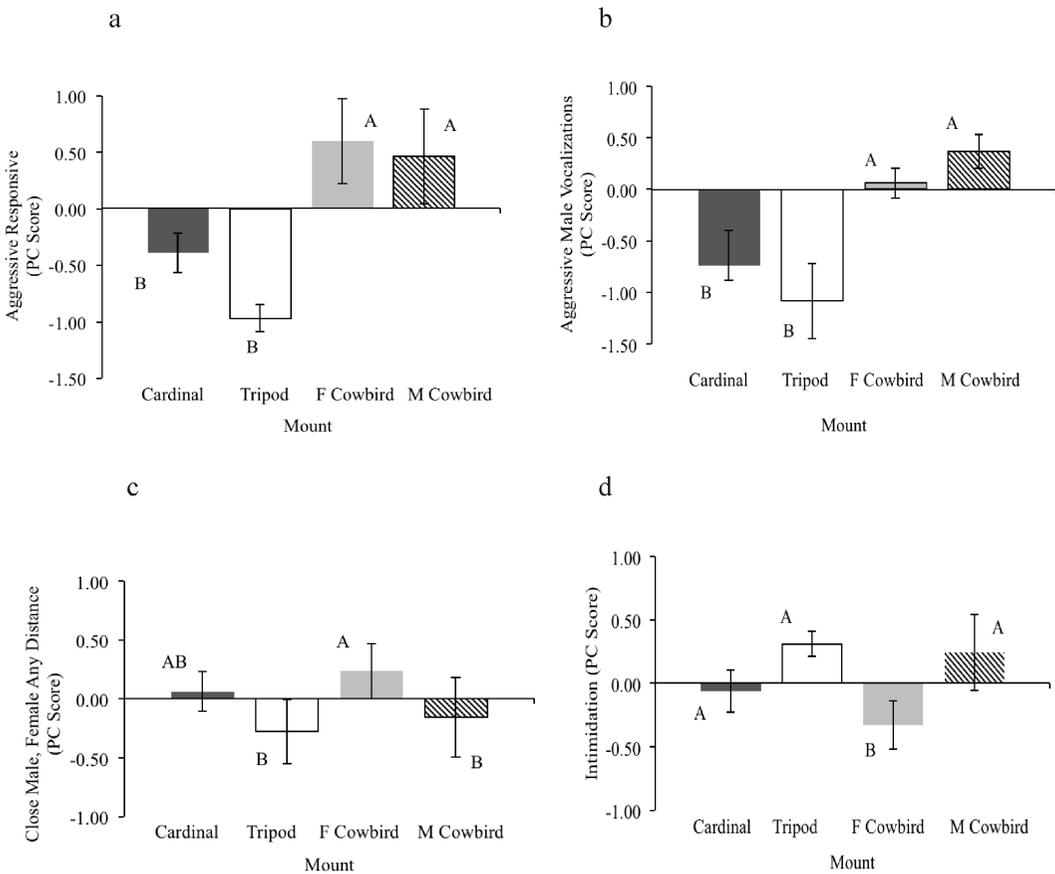
FIG. 2.   a–d. Differences in the responses (least square mean ± SE) of Red-winged Blackbirds (PC scores: ''Aggressive Response,'' ''Aggressive Male Vocalizations (sites with no known nests),'' ''Close Male, Female Any Distance,'' and ''Intimidation'') to all treatments of the mount and playback presentations. Mounts with different letters are *post hoc* significantly different from each other in the GLMM analysis. F: female, M: male.

similar between Nest and No Known Nest sites ($P > 0.05$).

Correlative Predictors.—The PC scores ''Male Aggressive Vocalizations'' were higher when redwings were defending known nests with nestlings than with eggs (Known Nest Data Only: $r^2 = 0.70$, $F_{1,9.41} = 10.035$, $P = 0.011$, Fig. 3b).

The following PCs had significantly higher scores at sites where the tripod with the model, and the playback speaker were placed closer to the nest than sites with model presentation farther away from the known nest (data not shown): ''Aggressive Response'' ($r^2 = 0.24$, $F_{1,14.41} = 7.07$, $P = 0.018$), ''Aggressive Female Vocalizations'' ($r^2 = 0.38$, $F_{1,13.38} = 4.79$, $P = 0.047$), ''Close Multiple Males and Multiple Females'' ($r^2 = 0.26$, $F_{1,14.08} = 8.41$, $P = 0.012$), and ''Close

Single Male, Single Female Any Distance'' ($r^2 = 0.24$, $F_{1,9.39} = 14.21$, $P = 0.004$).

DISCUSSION

Comparison 1.—Redwings displayed consistently more aggression toward both the male and female cowbird mounts than the female cardinal mount and the tripod. This suggests that the redwings respond to both male and female cowbirds as a different class of threat, relative to innocuous sympatric non-parasitic bird species and inanimate objects. Several previous studies (Robertson and Norman 1976, Strausberger and Horning 1998, Gill et al. 2008; summarized in S Table 1) found that behaviors of cowbird hosts toward male and female parasitic mounts were not significantly different, implying that either the
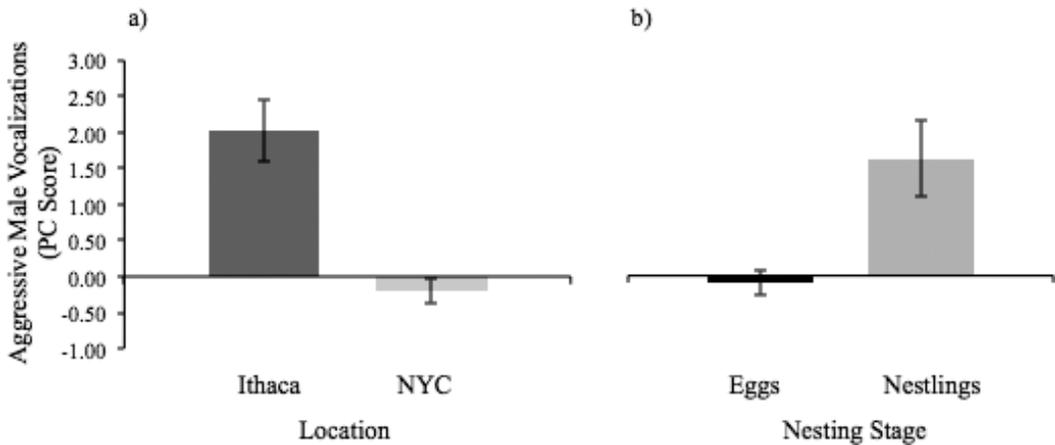
FIG. 3. a–b. Differences in the responses (least square mean ± SE) of Red-winged Blackbirds to all treatments of mount and playback presentations (PC scores: ''Aggressive Male Vocalizations'') between Ithaca and New York City (NYC), NY and between those defending nests and eggs. Only data from known nest sites was used in these analyses and plots. These differences were significant at $P < 0.05$.

redwings cannot distinguish the two sexes or that these hosts did not perceive the female cowbird as a unique threat relative to males. This conclusion is in line with the observation that even male Brown-headed Cowbirds may participate in nest searching behaviors attributed typically to females of this obligate parasitic species (Strausberger 1998, Igl 2003).

However, there also were several cowbird sex-specific differences in our host behavioral response data, similar to as seen in a different set of previous studies (Folkers 1982), in that redwing males positioned themselves closer to the female cowbird mount than toward the male cowbird mount and the two controls. These consistent mount-sex specific behavioral patterns imply that hosts respond to the different sexes of the brood parasite with different defensive strategies. Conversely, the PC ''Intimidation'' score was significantly less in response to the female cowbird mount than to the other two mounts and the tripod. This PC is positively correlated with the behaviors Dive, Hover, and No Reaction (S Table 3). A potential factor for why these behaviors were performed less often toward the female cowbird mount is perhaps that redwings were in fact more likely to physically strike the female cowbird mount than to dive and hover near it (Fig. 2d).

Comparison 2.—In Ithaca, throughout 5 years of research between 1997–2010, no parasitized redwing nests were found ($n > 100$ nests), even

though cowbird parasitism rates were consistently >20% per annum in other sympatric hosts (Hauber 2003b). In NYC, the first redwing nest in our 12 total nest sample was parasitized (8.3%) in 2010, but all other nests were not. Although we found that redwings produced significantly more aggressive vocalizations in Ithaca than in NYC (Fig. 1) in response to cowbird mounts, it remains unclear whether and how this may be related to variation in the risk of parasitism or the success to in fact reduce incidents of parasitism. Alternatively, it is possible that the redwings with territories inside public parks in NYC are habituated to a certain amount of disturbance and therefore do not react to all types of intruder stimuli as strongly as do these same hosts with territories in and around the private and rural Cornell Pond Units. Future studies should examine habitat and site-specific variation with larger sample sizes of redwing nests and their cowbird parasitism status across several urban-rural gradients (following Scales et al. 2011).

Comparison 3.—The only PC that was significantly different depending on whether there was a known active nest in the vicinity of the mount presentation was the ''Aggressive Male Vocalization'' PC. This PC was comprised of positive contributions from the vocalizations ''check,'' ''whistle,'' and ''ring'' (S Table 5); and redwings performed these behaviors significantly more often when defending known nests. Researchers looking to combine behavioral nest defense data

from redwings with or without known active nest sites would need to be aware of and account for this variability. Overall, however, we found that the behaviors from males without known nests were statistically similar to behaviors from redwings with known active nests. Our conclusion is that it is possible to combine data from nesting redwing pairs and redwing males without known nests to study these hosts' responses in cowbird mount and sound presentation experiments.

Correlative Predictors of Redwing Responses.— Although not as the outcome of an experimental design, we found statistical patterns indicating that redwings displayed more aggression toward the mounts when defending nestlings than eggs. Both female and male cowbirds are known to destroy host eggs and nestlings (Granfors et al. 2001, Igl 2003), perhaps as part of a farming or mafia strategy (Arcese et al. 1996, Hoover and Robinson 2007, Hauber 2009) and so, to examine whether redwings perceive male cowbirds as specific threats to nesting success, future mount presentation experiments should include larger sample sizes of redwing nests occupied by nestlings and compare reactions toward both sexes of cowbirds across nesting stages.

In addition, we found that aggressive responses were greater as the mounts and playbacks were displayed closer to the nests. Clotfelter (1998) also found that redwings were more aggressive towards a female cowbird mount as the distance from the mount to the nest decreased. Female cowbirds must be physically at the nest in order to remove host eggs and lay parasitic eggs, and so redwings would benefit from increasing their aggressive behaviors as female cowbirds approach so that the brood parasites do not land, access the nest, remove host eggs, and lay eggs of their own in the nest.

*Conclusions.—*Red-winged Blackbirds responded more aggressively toward mount and playback presentations of both sexes of the obligate brood parasite, the Brown-headed Cowbird, than to the female Northern Cardinal, and an empty tripod (as controls). In addition, male redwings approached the female cowbird presentation more closely relative to the male cowbird. These findings are in line with the theory that redwings mount nest defenses to reduce the cost of parasitism; indeed, Strausberger (2001) found an inverse association between host aggression toward a female cowbird mount and cowbird parasitism rates. Our study also compared antiparasitic aggressive behaviors of redwings be-

tween two sites in New York State, and found more aggression in redwings in rural Ithaca than in urban New York City. Finally, we compared the behaviors of the redwings with active nests to redwings with no known nests and documented that only one Principal Component, out of nine total PCs, differed significantly depending on whether there was a known active nest in the vicinity. This suggests that in an initial survey, the presence of an active host nest is not necessarily required to provide a suitable experimental analysis of redwings' responses toward taxidermic mounts and vocalization playbacks of brood parasitic cowbirds.

ACKNOWLEDGMENTS

LITERATURE CITED

Arcese, P., J. N. M. Smith, and M. I. Hatch. 1996. Nest predation by cowbirds, and its consequences for passerine demography. Proceedings of the National Academy of Sciences of the United States of America. 93:4608–4611.

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.

Bazin, R. C. and S. G. Sealy. 1993. Experiments on the responses of a rejector species to threats of predation and cowbird parasitism. Ethology 94:326–338.

Beletsky, L. D. 1996. The Red-winged Blackbird: the biology of a strongly polygynous songbird. Academic Press, San Diego, California, USA.

Briskie, J. V. and S. G. Sealy. 1989. Changes in nest defense against a brood parasite over the breeding cycle. Ethology 82:61–67.

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.

Burhans, D. E. 2001. Enemy recognition by Field Sparrows. Wilson Bulletin 113:189–193.

Burhans, D. E., B. M. Strausberger, and M. D. Carey. 2001. Regional variation in response of Field Sparrows to the threat of Brown-headed Cowbird parasitism. Auk 118:776–780.

Capper, C. L., M. F. Guigueno, and S. G. Sealy. 2012. Acceptance of simulated cowbird parasitism in a

northern population of Red-winged Blackbirds. American Midland Naturalist 167:127–135.

CLOTFELTER, E. D. 1998. What cues do Brown-headed Cowbirds use to locate Red-winged Blackbird hosts 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.

CRUZ, A. AND L. COOPER. 2001. Behavioral defenses against brood parasitism in the American Robin (*Turdus migratorius*). Final Report submitted to the City of Boulder Open Space Department, Colorado, USA, www-static.bouldercolorado.gov/docs/4177_Cruz_Alexander_Behavioral-1-201307091311.pdf (accessed 4 May 2010).

DAVIES, N. B. 2000. Cuckoos, cowbirds, and other cheats. London: T & AD Poyser, London, UK.

DAVIES, N. B. AND J. A. WELBERGEN. 2008. Cuckoo-hawk mimicry? Proceedings of the Royal Society B 275:1817–1822.

D'ORAZIO, K. A. AND D. L. NEUDORF. 2008. Nest defense by Carolina Wrens. Wilson Journal of Ornithology 120:467–472.

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.

FORSMAN, J. T. AND T. E. MARTIN. 2009. Habitat selection for parasite-free space by hosts of parasitic cowbirds. Oikos 118:464–470.

FREEMAN, S., D. F. GORI, AND S. ROWHER. 1990. Red-winged Blackbirds and Brown-headed Cowbirds: some aspects of a host-parasite relationship. Condor 92:336–340.

GILL, S. A., D. L. NEUDORF, AND S. G. SEALY. 1997. Host responses to cowbirds near the nest: cues for recognition. Animal Behaviour 53:1287–1293.

GILL, S. A., D. L. NEUDORF, AND S. G. SEALY. 2008. Do hosts discriminate between sexually dichromatic male and female Brown-headed Cowbirds? Ethology 114:548–556.

GILL, S. A. AND S. G. SEALY. 1996. Nest defense by Yellow Warblers: recognition of a brood parasite and an avian nest predator. Behaviour 133:263–282.

GILL, S. A. AND S. G. SEALY. 2004. Functional reference in an alarm signal given during nest defense: seet calls of Yellow Warblers denote brood-parasitic Brown-headed Cowbirds. Behavioral Ecology and Sociobiology 56:71–80.

GRANFORS, D. A., P. J. PIETZ, AND L. A. JOYAL. 2001. Frequency of egg and nestling destruction by female Brown-headed Cowbirds at grassland nests. Auk 118:765–769.

GRIM, T. 2005. Host recognition of brood parasites: implications for methodology in studies of enemy recognition. Auk 122:530–543.

GRIM, T. 2007. Experimental evidence for chick discrimination without recognition in a brood parasite host. Proceedings of the Royal Society B 274:373–381.

GRIM, T. 2011. Ejecting chick cheats: a changing paradigm? Frontiers in Zoology 8:14.

HALKIN, S. L. AND S. U. LINVILLE. 1999. Northern Cardinal (*Cardinalis cardinalis*). The birds of North America. Number 440.

HAUBER, M. E. 2000. Nest predation and cowbird parasitism in Song Sparrows. Journal of Field Ornithology 71:389–398.

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. 2003a. Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. Behavioral Ecology 14:224–235.

HAUBER, M. E. 2003b. Lower begging responsiveness of host versus parasitic Brown-headed Cowbird (*Molothrus ater*) nestlings is related to species identity but not to early social experience. Journal of Comparative Psychology 117:24–30.

HAUBER, M. E. 2009. Does the removal of avian brood parasite eggs increase host productivity? A case study with Brown-headed Cowbirds *Molothrus ater* and Song Sparrows *Melospiza melodia* near Ithaca, New York, USA. Conservation Evidence 6:83–88.

HAUBER, M. E., H. E. PEARSON, A. REH, AND A. MERGES. 2002. Discrimination between host songs by brood parasitic Brown-headed Cowbirds (*Molothrus ater*). Animal Cognition 5:129–137.

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.

HAUBER, M. E., S. A. RUSSO, AND P. W. SHERMAN. 2001. A password for species recognition in a brood parasite bird. Proceedings of the Royal Society of London B 268:1041–1048.

HAUBER, M. E., P. SAMAS, M. G. ANDERSON, J. RUTILA, J. LOW, P. CASSEY, AND T. GRIM. 2013. Life-history theory predicts host behavioural responses to experimental brood parasitism. Ethology Ecology and Evolution 26:(online).

HENGER, C. S., S. B. WALLACE, AND M. E. HAUBER. 2012. Comparison of two field data collection methods in recording avian behavior. Kingbird 62:198211.

HOBSON, K. A. AND S. G. SEALY. 1989. Responses of Yellow Warblers to the threat of cowbird parasitism. Animal Behaviour 38:510–519.

HONZA, M., V. ŠICHA, 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., 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.

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, USA 104:4479–4483.

HOSOI, S. A. AND S. I. ROTHSTEIN. 2000. Nest desertion and cowbird parasitism: evidence for evolved responses and evolutionary lag. Animal Behaviour 59:823–840.

IGL, L. D. 2003. Male Brown-headed Cowbird attacks and kills a nestling. Wilson Bulletin 115:210–212.

KACHIGAN, S. K. 1991. Multivariate statistical analysis: a conceptual introduction. Second Edition. Radius Press, New York, USA.

KENWARD, M. G. AND J. H. ROGER. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983–997.

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. 1988. Nest defense behavior in the Red-winged Blackbird. Condor 90:193–200.

LAHTI, D. C. 2006. Persistence of egg recognition in the absence of brood parasitism: pattern and mechanism. Evolution 60:157–168.

LANGMORE, N. E., S. HUNT, AND R. M. KILNER. 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. Nature 422:157–160.

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.

LOTEM, A. 1993. Learning to recognize nestlings is maladaptive for cuckoo *Cuculus canorus* hosts. Nature 362:743–745.

LOWTHER, P. E. 1993. Brown-headed Cowbird (*Molothrus ater*). The birds of North America. Number 47.

MARK, D. AND B. J. STUTCHBURY. 1994. Response of a forest interior songbird to the threat of cowbird parasitism. Animal Behaviour 47:275–280.

MARTIN, P. AND P. BATESON. 2009. Measuring behavior: an introductory guide. Cambridge University Press, New York, USA.

MOSKÁT, C. AND M. E. HAUBER. 2007. Conflict between egg recognition and egg rejection decisions in Common Cuckoo (*Cuculus canorus*) hosts. Animal Cognition 10:377–386.

MOSKÁT, C., A. ZÖLEI, M. BAN, Z. ELEK, L. TONG, N. GELTSCH, AND M. E. HAUBER. 2014. How to spot a stranger's egg? A mimicry-specific discordancy effect in the recognition of parasitic eggs. Ethology 120:616–626.

NEUDORF, D. L. AND S. G. SEALY. 1992. Reactions of four passerine species to threats of predation and cowbird parasitism: enemy recognition or generalized responses? Behaviour 123:84–105.

O'HARA, R. B. 2009. How to make models add up – a primer on GLMMs. Annales Zoologici Fennici 46:124–137.

ORTEGA, C. P. AND A. CRUZ. 1988. Mechanisms of egg acceptance by marsh-dwelling blackbirds. Condor 90:349–358.

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.

ORTEGA, J. C., C. P. ORTEGA, AND A. CRUZ. 1993. Does Brown-headed Cowbird egg coloration influence Red-winged Blackbird responses towards nest contents? Condor 95:217–219.

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.

RAND MCNALLY. 2004. The road atlas: United States, Canada, Mexico. Skokie, Illinois, USA.

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.

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.

ROTHSTEIN, S. I. 1975. 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.

ROWHER, 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.

SCALES, J., J. HYMAN, AND M. HUGHES. 2011. Behavioral syndromes break down in urban Song Sparrow populations. Ethology 117:887–895.

SEALY, S. G., D. L. NEUDORF, K. A. HOBSON, AND S. A. GILL. 1998. Nest defense by potential hosts of the Brown-headed Cowbird: methodical 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, New York, USA.

SEARCY, W. A. AND K. YASUKAWA. 1995. Polygyny and sexual selection in Red-Winged Blackbirds. Princeton University Press, Princeton, New Jersey, USA.

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. 1998. 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.

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 1:20–27.

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.

WESTNEAT, D. F. 1992. Nesting synchrony by female Red-winged Blackbirds: effects on predation and breeding success. Ecology 73:2284–2294.

YASUKAWA, K. 1981. Song and territory defense in the Red-winged Blackbird. Auk 98:185–187.

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., 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.