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Why Do Brown-headed Cowbirds, (Molothrus ater), Attack Eggs in Host Nests?

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of Master
of Science.

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ABSTRACT

Although Brown-headed Cowbirds (Molothrus ater) have been observed to remove and/or damage eggs in host nests, little work has been done to identify factors that influence the frequency and extent of these attacks on eggs or to investigate the adaptive value of this behavior. Two hypotheses have been proposed to explain the occurrence of cowbird egg attacks: 1) the predation hypothesis, which assumes that cowbirds utilize eggs as an alternative food source; and 2) the breeding strategy hypothesis, which suggests that cowbirds benefit from attacks on eggs because the removal and/or damage of eggs improves the chances of successful parasitism. The purpose of this study was to test the two hypotheses by examining the effects of various ecological factors on cowbird tendency to remove and/or damage eggs. In addition, the effects of exogenic prolactin and testosterone were investigated to determine whether this behavior is controlled hormonally.

The results of experiments designed to examine the role of ecological factors on cowbird responses to eggs indicated that cowbird tendency to attack eggs was restricted to breeding individuals and was influenced by the number and condition of the eggs in the nests. Furthermore, food shortage did not seem to be the primary cause for egg attacks because the contents of attacked eggs were not usually consumed and the duration of food deprivation did not influence the frequency of attacks. These findings provide strong support for the breeding strategy hypothesis and argue against the predation hypothesis.

Four functions were proposed for egg removal as a breeding strategy: A) create space for cowbird eggs in host nests; B) to reduce competition among nestlings for parental care; C) to force renesting by hosts when nests are located too late for successful placement of cowbird eggs; and/or D) to induce renesting by hosts to promote asynchrony among hosts and extend the period when foster nests are available to cowbirds. Because clutch size influenced the proportion of cowbirds that responded but availability of nests did not, cowbird egg attacks appeared to be linked only with the first three functions.

Although adult cowbirds stopped attacking eggs in late July, around the time that cowbirds generally finish breeding, cowbirds of both sexes and all ages tended to remove and/or damage eggs during experimental trials in September and October. This cyclic pattern in cowbird responses to eggs suggests that, like many of the behaviors associated with reproduction, the cowbird egg attack behavior is hormonally influenced. In order to test this idea more directly, I artificially increased the levels of prolactin and testosterone in captive cowbirds. The trials revealed that, in females, the addition of exogenic testosterone promoted egg attacks and the addition of prolactin inhibited egg attacks. Whereas male cowbird tendency to remove and/or damage eggs was not influenced by the experimental manipulation of either hormone. Although this study was not successful in demonstrating that prolactin and testosterone directly control male cowbird egg-pecking tendency, the effects of these hormones on female cowbird responses to eggs, along with the observation that all cowbirds failed to respond to eggs during molt, suggest that cowbird egg attack behavior is hormonally controlled.

GENERAL INTRODUCTION

Brown-headed Cowbirds, Molothrus ater, lay their eggs in the nests of other birds and leave the care and rearing of their young to the "foster" parents (Friedmann 1929). This strategy, known as interspecific brood parasitism, provides some obvious benefits. Because the number of offspring produced is not limited by the need to provide parental care, the time and energy normally reserved for such tasks can be used to produce more eggs. As well, brood dispersal decreases the likelihood that all offspring will be lost to predation or starvation (Friedmann 1929, 1963).

Brown-headed Cowbirds are obligate parasites (Friedmann 1929). Hence, even when no suitable host nests are available, cowbirds cannot resume parental duties. Given this dependence on hosts, it might be expected that cowbirds would specialize with one or two species to maximize their chances of successful parasitism. However, the generalist strategy which they have adopted for reproduction provides them with a number of important advantages. Because brood parasitism typically reduces the reproductive success of the host species (Klaas 1975, Mayfield 1977, Elliott 1978), the cowbird practice of spreading the ill-effects over a number of hosts may increase the period of time which elapses before counter measures such as ejection or burying of the eggs and nest desertion are adopted by the hosts (Norman and Robertson 1975). As well, the cowbird's ability to parasitize a large number of hosts enables it to enlarge its range and improve the likelihood of encountering additional host species (Friedmann 1963).

Other adaptations also enhance cowbird success as a generalist brood parasite. For instance, male cowbirds do not undergo testes regression until late in July, and so are able to produce sperm throughout the breeding season (Scott 1963). Similarly, females are able to lay eggs almost continuously from early May until late in July, with no ovary regression between clutches and a greatly reduced resting time between laying sequences (Scott and Ankney 1980). This expansion of the cowbird egg laying period is accompanied by an increase in egg producton; females are able to produce approximately 40 eggs over their eight week breeding period (Scott and Ankney 1980).

As for the success of cowbird eggs in host nests, it is influenced by a number of factors including the acceptance of these eggs by hosts, the ability of hosts to feed and care for the cowbird young, the timing of cowbird egg placement relative to host laying, and the success of cowbird nestlings when competing for parental care. Interestingly, cowbirds show no inclination towards mimicry of host eggs or nestlings and appear to place their eggs in the nests of sympatric species indiscriminately (Friedmann 1963). Also, in contrast to many other brood parasitic species, cowbird chicks do not damage or evict any eggs or nestlings (Blankspoor et al 1982). Adult cowbirds, on the other hand, frequently do remove and/or damage eggs in host nests (Friemann 1929, Nice 1937, Hann 1941, Norris 1947, Mayfield 1960). Two hypotheses have been proposed to explain the occurrence of these egg attacks: 1) the predation hypothesis, which assumes that cowbirds utilize eggs as an alternative food source, and 2) the breeding strategy hypothesis, which suggests that cowbirds benefit from attacks on eggs because the removal and/or damage of eggs improves the chances of successful parasitism (Freidmann 1963). However, despite the possible association between the egg attack behavior and the reproductive strategy of cowbirds, little work has been done to investigate the adaptive value of cowbird attacks on eggs.

Data on cowbird egg attacks have, in the past, arisen primarily from two sources: from direct observation of cowbird activities at host nests, and from studying the relative clutch sizes of host nests with and without cowbird eggs. Direct observations of cowbirds are extremely difficult to make, due to the large distances travelled by individuals and to the fact that reproductive behaviors of cowbirds are spread among a large number of host nests (Rothstein et al. 1984). Information obtained from relative clutch sizes is also unsatisfactory because it is based on the assumption that all nests disturbed by cowbirds are subsequently parasitized. Furthermore, information on the removal of host eggs is insufficient to test the predictions of the two above hypotheses).

In order to test the predation and breeding strategy hypotheses, cowbird responses to eggs have to be examined under controlled conditions. I chose, therefore, to conduct my studies with captive birds. This approach made it possible to test the effects of a number of factors on egg attack behavior that could not be investigated under natural conditions.

The purpose of this study is to test the two hypotheses proposed to explain the occurrence of cowbird egg attacks. This will be achieved by examining the effects of various ecological factors on cowbird tendency to remove and/or damage eggs (chapter one). If egg attacks are a specific breeding strategy, it is possible that, like many behaviors associated with reproduction, egg attacks are controlled hormonally. In chapter two, I will investigate the possible hormonal basis of the cowbird tendency to attack eggs by examining cowbird post-breeding responses to eggs and the effects of exogenic prolactin and testosterone on cowbird tendency to attack eggs.

CHAPTER 1

ECOLOGICAL FACTORS INFLUENCING THE FREQUENCY AND EXTENT OF COWBIRD ATTACKS ON EGGS

INTRODUCTION

Egg removal by Brown-headed Cowbirds (Molothrus ater) has been reported a number of times (Friedmann 1929, Nice 1937, Hann 1941, Norris 1947, Mayfield 1960, 1961, 1977, Friedmann 1963, Friedmann et al. 1977, Payne 1977, Clark and Roberson 1981, Blankespoor et al. 1982, Murphy 1986, Burgham and Picman unpublished data.). Little work has been done, however, to determine what factors influence the frequency and extent of cowbird egg attacks. Since the cowbird tendency to remove and/or damage eggs in host nests appears to be widespread despite its associated costs (e.g. time, energy, and risk of aggression from hosts), egg attacks must confer some benefit on cowbirds or their offspring. Two hypotheses may be proposed to explain how egg attacks could be beneficial. The first hypothesis suggests that as eggs are rich in protein and calcium, eggs could act as an alternative food source (henceforth referred to as the predation hypothesis). Female cowbirds produce approximately forty eggs during the eight-week breeding period without significantly reducing their protein or fat reserves (Ankney and Scott 1980, Scott and Ankney 1983). During this time, the cowbird's diet is shifted from mainly seeds to mainly insects, demonstrating an increased need for protein (Ankney and Scott 1980). As well, cowbirds consume mollusk shells which are high in calcium (Ankney and Scott 1980). This need to acquire resources to produce eggs suggests that females especially, may utilize eggs as an alternate food source. The second hypothesis proposes that egg attacks could be a mechanism for increasing the chances of successful parasitism (henceforth referred to as the breeding strategy hypothesis). This could be achieved if egg removal and/or damage: A) increases the space for cowbird eggs in host nests; B) reduces competition among nestlings for parental care; C) allows the laying of a cowbird egg at the appropriate time relative to host laying by forcing host renesting when nests are found after host incubation has been initiated; and/or D) promotes nesting asynchrony among hosts to extend the period when host nests are available to cowbirds.

Due to difficulties in studying wild cowbirds (e.g. very large home ranges, reproductive activites divided among many nests, etc.), the information available to test the two hypotheses on the adaptive value of cowbird egg attacks is limited. Data from a small number of direct observations indicated that female cowbirds often removed one or more eggs from host nests, usually on or before the day they intended to lay their own egg and that the contents of attacked eggs were frequently consumed (Nice 1937, Hann 1941). Predation was not thought to be the main purpose for these attacks on eggs because attacks appeared to be i) restricted to parasitized nests, ii) limited to nests containing more than one egg, and iii) carried out only by female cowbirds (Friedmann 1929, Nice 1937, Hann 1941). However, male interest in nests, including observations of egg destruction, also has been documented recently (Mengel and Jenkinson 1970, Rothstein et al. 1984, Burgham and Picman unpublished data).

Although the proposed reasons for egg attacks need not be mutually exclusive, their influences on the pattern of cowbird egg attacks and the extent of damage done to eggs are likely to be different. The predation hypothesis predicts that: 1) the contents of eggs should typically be consumed; 2) the tendency to attack eggs should be greatest when food demands are high; 3) egg attacks should continue whenever eggs are available; and 4) that this behavior should be exhibited by all individuals, regardless of their age and sex. In addition, this hypothesis predicts that 5) cowbird tendency to attack eggs should be influenced neither by clutch size, nor 6) by the number of available nests since any nest(s) with at least one egg would represent a potential food source. However, 7) the number of eggs damaged in a given attack may increase with the total number of eggs presented to some finite point, corresponding to the cowbird's satiation point. The predation hypothesis also predicts that cowbirds should respond to broken eggs and, if the eggs are fresh, should consume their contents (prediction 8). Finally, the overall tendency to remove and/or damage eggs should be independent of the size and type of eggs present (i.e. acceptor host versus rejector host) (predicton 9).

Alternatively, if egg removal is a specific reproductive strategy associated with brood parasitic activities (hypothesis two), then the following predictions can be made: 1) the content of eggs need not be consumed, and hence, 2) the length of time without food should not affect cowbird responses to eggs. Since interest in nests and eggs will be a consequence of breeding activities, egg attacks should be restricted to the breeding season (prediction 3) and to breeding individuals (prediction 4). Cowbird responses to eggs should be influenced by clutch size and the number of nests available (predictions 5 and 6, respectively), although the effects of these factors should differ among the four proposed functions of egg attacks. The frequency of cowbird egg attacks should increase with clutch size if removal and/or damage to eggs leads to more space for cowbird eggs in host nests (function A), reduced competition among nestlings for parental care (function B) or allows the laying of a parasitic egg at the appropriate time relative to host laying (function C). These benefits from clutch size reduction should not be influenced by the number of nests present. On the other hand, if egg attacks promote asynchrony among hosts (function D), then the frequency of positive responses should increase with increasing nest density but should not be affected by clutch size. Another prediction of the reproductive strategy hypothesis concerns the patterns of damage inflicted on eggs. Functions A and B require that cowbird activities be discreet so as to minimize adverse effects on hosts and hence the chances of their abandonment of the nests. As a consequence, the breeding strategy hypothesis predicts, that if egg attacks fulfill functions A and/or B, then only a small, finite number of eggs should be damaged per nest (prediction 7A,B). Conversely, to fulfill functions C and D, cowbird activities must promote the disruption of host nesting attempts. In these cases, damage should be extensive and may frequently involve destruction of complete clutches (prediction 7C,D). Because passerines usually evict damaged eggs from their nests (Rothstein 1982), broken eggs should not ellicit positive responses by cowbirds (prediction 8). Attacks might continue, however, if only some eggs in nests were damaged. Cowbirds are known to parasitize over 200 species, although some hosts are parasitized more frequently than others (Friedmann 1963). This difference could reflect cowbird preference for certain species or may be a consequence of unequal nest accessibility caused by differences in the relative abundance of hosts and/or different location of nests or host nest defense (Friedmann 1963). If cowbirds are able to discriminate among various types of eggs, then the reproductive strategy hypothesis would also predict that the eggs of rejector species may be removed and/or damaged less frequently than those of acceptor species (prediction 9).

The purpose of this chapter is to test the predictions proposed by the two hypotheses to explain the occurrence of egg removal and/or damage by cowbirds (see Table 1 for a summary of these predictions). In order to achieve this, I will examine the effects of food availability, time in season, clutch size, number of nests, total number of eggs, egg condition, and egg type on the responses of captive cowbirds to nests with eggs.

METHODS

General Procedures

A total of 101 Brown-headed Cowbirds (32 males, 28 females and 41 juveniles) were captured in the Mer Bleue Bog Conservation area just outside Ottawa, Ontario, between June 1st and August 20th, 1987, and between May 10th and July 6th, 1988, using spring loaded traps placed on feeding stations. Birds were transported to the University of Ottawa animal care facilities where they were weighed, measured (wing and tarsus length), and fitted with colored leg bands for individual identification. Cowbirds were housed in individual cages (60cm x 45cm x 60cm) which prohibited visual contact between neighbours. Food (wild bird seed) and water were available *ad libitum*. Birds were kept on a 16:8 light:dark cycle until late August when the cycle was gradually changed to 12L:12D.

Throughout the study, birds were presented with artificial nests containing one or more eggs once per day (a description of the testing procedure is given below). Artificial nests were constructed by pressing two layers of grass, with a few strips of transparent LePage glue in between, into plastic bowls the size and shape of a Song Sparrow nest, *Melospiza melodia*, nest (9-14 cm; averaged sized host nest). Blue-breasted Quail, *Coturnix chinensis*, eggs were used for most trials as they are similar in size (24 mm x 18 mm) to those of many host species known to be parasitised by cowbirds (e.g. Red-winged Blackbird, 25 mm x 18 mm; Ovenbird *Seiurus aurocapillus*, 20 mm x 15 mm; Red-Eyed Verio, *Vireo olivaceus*, 20 mm x 14 mm; Cedar Waxwing, *Bombycilla cedrorum*, 22 mm x 16 mm; Eastern Kingbird, *Tyrannus tyrannus*), and were available in the quantities required for the study. As the shells of the quail eggs were much thicker than those of the natural hosts, the eggs were treated with acetic acid (i.e. eggs were placed in a 20% acetic acid solution for 20 minutes and then thoroughly rinsed in running water). This procedure does not affect the palatability of the eggs for predators, such as Meadowlarks (*Sturnella magna*, S. *neglecta*), Blue Jays (*Cyanocitta cristata*), Crows (*Corvus brachyrhynchos*), Raccoons (*Procyon lotor*), Thirteen-lined Ground Squirrels (*Citellus tridecemlineatus*), and Stripped Skunks (*Mephitis mephitis*) (Picman 1987, Schaeff and Picman unpublished data). Other eggs used in the study (where indicated) included Common Quail, *Coturnix coturnix*, (also treated with acetic acid), Yellow-headed Blackbird, and Red-winged Blackbird eggs.

Each trial consisted of placing one or two nests containing one or more eggs in a wire holder attached to the wall of the appropriate bird's cage such that the bottom of the nest was touching the floor. Nests were left in the cages for three hours during which time behavioral events were recorded using a video camera placed three meters from the cage. The birds' responses to the nests and eggs during the first ten minutes after presentation were evaluated according to the following factors: time to approach the nests, the number of approaches made, the number of times the nest was entered, and whether the eggs were pecked and/or removed. Eggs and nests were removed and scored for damage after each three-hour test was completed. Damage evaluation included the location of the egg, the number and size of holes in the egg, and the amount and condition of the remaining yolk and white. The volume of egg contents present was estimated visually for all trials except the food deprivation experiment, where the eggs were weighed before and after presentation.

Before beginning trials to test the predictions of the two proposed hypotheses, cowbirds were presented with single nests containing one egg once per day at 05:30, 09:30, 13:30, and 17:30 hours. Male and female cowbird responses to eggs were similar at the four test times (Table 2). However, for consistency, all subsequent trials were initiated between 16:00 and 18:00 hours (except where otherwise indicated).

Throughout this study, I examined the effects of a number of factors on cowbird responses to nests and eggs. The experimental treatments are described below. Where the predicted effects were directional, the results were analysed using one-tailed tests.

Effect of Food Deprivation

To test predictions 1 and 2 (see Table 1) cowbirds were denied access to food (but not water) for 0, 8, 13, and 32 hours prior to the presentation of single nests containing one egg. Food was removed at 05:00 for each specified period of time. As a result, behavioral testing was initiated at 13:00 for control 1 (0 hours), 8-hour treatment, and 32-hour treatment, and at 18:00 for control 2 (0 hours), and 13-hour treatment. For these trials, eggs were placed in small white weighing cups and weighed before and after presentation to facilitate a more accurate measurement of changes in egg contents. The presence of the cups did not affect the rate of cowbird responses (i.e. 38% of birds attacked eggs during trials with cups as compared to 48% of cowbirds during trials without cups; $X^2=0.53$, d.f.=1, $p>0.5$). To determine whether the periods of food deprivation were sufficient to influence the cowbirds' feeding patterns, four cowbirds were presented with food and eggs following eight hours without food. Upon presentation of food, all four individuals went immediately to the food dishes and fed the following five to 10 minutes.

Effect of Time in the Season and Age and Sex of Cowbirds

To test predictions 3 and 4 (see Table 1), adult cowbirds were tested from May (pre-laying) through July (post-breeding), and juveniles from June (after fledgling) through July. During these trials, one quail egg was placed in a single experimental nest.

Effect of Clutch Size, Number of Nests, and Overall Abundance of Eggs

To test predictions 5, 6, and 7 (see Table 1) cowbirds were presented with 1 and 2 nests containing 1, 3, or 5 eggs. During multiple nest trials, the number of eggs was the same in both nests and nests were positioned in opposite corners at one end of the cage. The order of presentation of nests with different clutch sizes was randomized and these trials were alternated with trials testing the effect of egg condition (see below).

Effect of Egg Condition

To test prediction 8 (see Table 1), cowbirds were presented with single nests containing 1) one undamaged egg, 2) one damaged egg, 3) five undamaged eggs, and 4) 5 eggs of which one, three or five were damaged. 'Damaged' eggs were created by punching a small hole (approximately 2.5 x 2.5 mm) in fresh quail eggs. These eggs were then positioned in nests so that the damaged portion of the egg was facing up. The order of presentation of nests with damaged eggs was randomized and these trials were alternated with trials testing the effects of clutch size (see above).

Effect of Egg Type

Prediction 9 (see Table 1) was tested by comparing cowbird responses to four types of eggs (Red-winged Blackbird, Yellow-headed Blackbird, Common Quail and Blue-breasted Quail) which differed in size, color, and host type (i.e. acceptor versus rejector) (Table 3). Each type was presented separately in nests containing a single egg.

RESULTS

Cowbirds were considered to have responded positively to eggs if at least one egg had been pecked and/or removed from the nests during the three hours of presentation. Initially, cowbirds tended to remove eggs as opposed to leaving damaged eggs in the nests. After ten trials, however, most individuals of both sexes had stopped removing eggs (Table 4). Egg removal was not always accompanied by egg breakage (in initial removals 40% of eggs were removed without breakage, in later trials 22% were removed without breakage; $X^2=1.09$, $p>0.25$).

The damage inflicted to eggs varied from trial to trial and consisted of almost invisible incomplete holes made with the tip of the beak, small holes less than 2 mm in diameter, medium holes approximately 3 mm in diameter, and large holes (greater than 5 mm in diameter), where as much as one half of the shell could be missing. One or more eggs received obvious damage (i.e. more than 2 mm^2 of shell damaged overall) in 60% to 85% of all trials with single nests, regardless of clutch size.

Individuals who attacked eggs were more likely to approach the nests during the ten minute observation period immediately following egg presentation than were individuals that did not attack eggs (e.g. for single nest trials with one egg: 50% of responsive individuals approached as compared to 13% of unresponsive individuals; $X^2=10.03$, $p<0.005$). Similarly, the average number of trips to nests made during the ten minute study period was higher for responsive individuals (1.7 [± 2.2]) as compared to unresponsive individuals (0.32 [0.88]; Mann-Whitney U-test, $p<0.001$).

Egg contents did not appear to be consumed by either sex in most trials, regardless of whether the eggs presented were treated with acetic acid (i.e. quail) or were natural (i.e. blackbird) [25% (N=24) of untreated eggs were consumed, compared to 15% (N=38) of treated eggs; $X^2=0.77$, $p>0.25$]. This suggests that the acetic acid treatment did not interfere with the cowbird responses to quail eggs. It was difficult to determine how much, if any, of the egg contents were missing in many cases because eggs were frequently moved or tipped over after having been damaged and their contents spilt (see the section on the effects of food deprivation). When eggs were consumed, some yolk and white were usually taken (78%), as well as up to one-third of the shell was consumed.

The tendency to attack eggs for cowbirds tested within two days of capture was similar to that of birds held in captivity for seven to ten days prior to testing (for females: $X^2=0$, d.f.=1, $p>0.9$; for males: $X^2=2.05$, d.f.=1, $p>0.5$). The rate of response was also similar among individuals caught and tested in different years (for females: $X^2=0.0$, d.f.=1, $p>0.99$; for males: $X^2=2.05$, d.f.=1, $p>0.1$). Therefore, all data from the two years were combined in the following analyses.

Effect of Food Deprivation

Female and male cowbirds did not usually consume the contents of attacked eggs, regardless of whether food was available *ad libitum* or had been removed for up to 32 hours (Tables 5 and 6). Similarly, the duration of food deprivation had no effect on the frequency of male or female cowbird attacks on eggs. Contrary to predictions 1 and 2 of the predation hypothesis, these results suggest that hunger is not the primary motivation for cowbird egg attacks (see Table 1).

Effect of Time in Season

The proportion of cowbirds that responded positively to nests with eggs differed throughout the year (Table 7). From May to the end of July approximately 50% of adult males and females reacted positively to nests containing a single egg. The response level for both sexes then dropped to approximately 10% in late July/early August, coinciding with the time when cowbirds typically finish laying and begin molt. Because juvenile cowbirds are frequently difficult to sex (Baird 1958), all juveniles were grouped together. When presented with experimental eggs and nests, juveniles frequently approached the nests, however, they seldomly pecked or removed the egg (Table 7).

All females tested responded positively to nests and eggs at least twice during the testing period (N=24), whereas several males failed to attack eggs even upon presentation of nests with five egg clutches (5 out of 38 males [13%] were unresponsive; comparison between the sexes: $X^2=3.34$ d.f.=1, $p>0.05$). The unresponsive individuals were captured at feeding stations from flocks consisting entirely of males; three in late July, 1987 and two in late June 1988. Individuals captured in 1987 were not examined for breeding status, but it was determined that those captured in late June, 1988 were not sexually active (i.e. their cloaca was not extended). Rothstein et al. (1987) also observed that individuals that were engaged in little or no breeding fed more frequently at artificial feeding stations.

These results suggest that egg attack behavior is restricted to the breeding season and to breeding individuals, as predicted by the breeding strategy hypothesis (see Table 1, predictions 3,4).

Effect of Clutch Size and Number of Nests Available

FEMALES: The proportion of females that attacked eggs was significantly higher for trials with 5 egg clutches as opposed to 3 or 1 egg clutches (Table 8). However, there was no difference in the level of response to a given clutch size when 1 or 2 nests were presented (Table 8). Similarly, although the number of eggs damaged by females increased with the total number of eggs presented, the average number of eggs damaged per nest for a given clutch size remained the same for one and two nest trials (Table 9). Responses involving complete clutch destruction were uncommon for both three and five egg clutch trials (21% of 3 egg clutch trials with 1 or 2 nests; 9% of 5 egg clutch trials with 1 or 2 nests; $X^2=1.3$, $p>0.25$). These patterns in female responses to eggs support predictions 5, 6, and 7 of the breeding strategy hypothesis while contradicting predictions 5-7 of the predation hypothesis (see Table 1). Furthermore, the lack of effect of multiple nest presentation suggests that egg attacks fulfill the functions of A, B, and/or C rather than D. The data on damage inflicted to eggs does not permit additional discrimination between the proposed functions.

MALES: Although the proportion of males that attacked eggs increased with clutch size, the difference was significant for single nest trials only. There was no difference in the level of response to a given clutch size for 1 and 2 nest trials (Table 8). The egg damage inflicted by male cowbirds did not differ significantly from that inflicted by females for any given set of trials. However, male damage patterns appeared to be influenced by the overall abundance of eggs rather than the number of eggs present in each nest (i.e. clutch size) (Table 10). The number of eggs damaged by males increased with the total number of eggs presented up to a total of 5 eggs. The average number of eggs destroyed then remained constant, irrespective of the clutch size or the number of nests presented. Responses involving complete clutch destruction were uncommon (44% of 3 egg trials with 1 or 2 nests, 19% of 5 egg clutch trials with 1 or 2 nests; $X^2=2.93$, $p>0.05$). These patterns of male egg attacks support predictions 5 and 6 of the breeding hypothesis and contradict those (5 and 6) of the predation hypothesis (see Table 1). The pattern for damage inflicted to eggs by cowbirds (predicton 7) lends support to the predation hypothesis.

Effect of Egg Condition

Male and female cowbirds responded significantly less often to nests with 5 and 1 egg clutches when one or more eggs were damaged prior to presentation (Table 11). The proportion of individuals that responded was similar, regardless of whether 1, 3, or 5 eggs were damaged in 5 egg clutches. This finding supports prediction 8 of the breeding strategy hypothesis but contradicts that of the predation hypothesis (see Table 1).

Effect of Egg Type

Because male and female responses to eggs were similar, data on the effect of egg type were combined. There were no significant differences in cowbird tendency to attack eggs on the basis of color, size, or host status (i.e. acceptor species versus rejector species, Table 12). However, the proportion of blackbird eggs that were removed rather than attacked and left in the nests, was high given that these birds had all been tested more than 15 times. The frequency of removal of redwing eggs was significantly higher than that for quail eggs during the later trials [i.e. after more than ten experimental trials] (X^2=6.31, d.f.=1, p<0.025). The rate was similar to that observed during the initial quail trials [first ten experimental trials] (X^2= 0.0, d.f.=1, p>0.99). The frequency of removals for yellowhead eggs did not differ significantly from either the initial or subsequent trial controls with quail eggs (early trials: 62% of eggs were removed, X^2=0.95 d.f.=1 p>0.25; later trials: 20% of eggs were removed; X^2=1.78 d.f.=2, p>0.1).

The lack of an effect of egg type on cowbird tendency to attack eggs is consistent with prediction 9 of the predation hypothesis (see Table 1). The increase in removals but not the overall tendency to attack eggs further suggests that cowbirds might be able to discriminate between egg types (i.e. cowbirds were reacting to the blackbird eggs as a novel stimulus). Hence, the lack of an effect of egg type also contradicts prediction 9 of the breeding strategy.

DISCUSSION

Two hypotheses were proposed to explain cowbird attacks on eggs in host nests; 1) the predation hypothesis, which assumes that cowbirds utilize eggs as an alternative food source; and 2) the breeding stategy hypothesis, which suggests that cowbirds benefit from attacks on eggs because the removal and/or damage of eggs improves the chances of successful parasitism. Although these two hypotheses need not be mutually exclusive, the results from this study tend to support the breeding strategy hypothesis while arguing against the predation hypothesis.

The contents of attacked eggs were not consumed in most trials, even when cowbirds had been denied access to food for up to 32 hours. Similarly, the proportion of cowbirds that removed and/or damaged eggs did not increase following food deprivation. Although captive conditions may have eventually altered cowbird need for special nutritional requirements (e.g. calcium), the initial trials for most females occurred the day after laying, when their resource reserves should have been depleted. Hence, the periods of food deprivaton should have created a substantial need for protein and other nutrients necessary for basic sustenancence. Thus, if eggs were a regular part of most cowbirds' diet, then food deprivation should have promoted attacks on eggs. The lack of an effect of food shortage on cowbird tendency to attack eggs provides the strongest evidence against the predation hypothesis.

Adult cowbirds of both sexes stopped removing and/or damaging eggs in late July, coinciding with the end of cowbird breeding. Although the availability of nests would normally be much lower this late in the season, if eggs represent an important source of protein and nutrients, then cowbird attacks on eggs should be maintained during this period when energy demands are usually high due to the initiation of post-breeding molt and pre-migratory body fat build-up (Friedmann 1929).

My results indicate that male cowbird tendency to attack eggs is only slightly less than that of females, yet reports of male interest in nests are very rare (Mengel and Jenkinson 1970, Rothstein et al. 1984, Burgham and Picman unpublished data). Interactions between male and female cowbirds may influence the frequency of male egg attacks, especially since attacks appear to be restricted to parasitized nests (Friedmann 1929, Hann 1941, Schaeff unpublished). Because of this, the presentation of nests to isolated male cowbirds may not have been representative of normal circumstances (i.e. attacks by males may be less frequent under normal conditions).

Not all male cowbirds may breed in a given season due to a skewed sex ratio (the ratio of males to females 1.6:1, Dufty 1982; 1.5:1, Darley 1971; 1.3:1, Teather and Robertson 1986; 1.1:1, Elliott 1980) and a tendency, in at least some populations, for polygynous matings (Payne 1973, Darley 1982, Rothstein et al. 1984, Teather and Robertson 1986). The failure of several males-- some of whom were obviously not sexually active-- and most juveniles to attack experimental eggs, therefore, suggests that this behavior may be restricted primarily to breeding individuals. This restriction, coupled with the abrupt change in cowbird responses to eggs at the end of cowbird egg laying, indicates that egg attacks may be hormonally controlled.

Both male and female cowbirds were more likely to attack eggs during trials with complete rather than incomplete clutches, but there was no difference in the number of individuals that responded for a given clutch size when two, rather than one, nests were presented. This dependency on clutch size, rather than the absolute number of eggs or nests available, supports the predictions of three of the four functions proposed for the breeding stratagey hypothesis (i.e. egg removal and/or damage may: (A) create space for cowbird eggs in host nests, (B) reduce competition among nestlings for parental care, and (C) adjust the timing of host laying). However, this finding is inconsistent with the predictions of the fourth function-- that egg attacks may promote laying asynchrony among hosts, and the predation hypothesis, both of which predict that nests with different numbers of eggs should influence cowbird tendency to attack eggs equally. The need to promote nesting asynchrony among hosts implies that the number of host nests available at a given time exceeds the amount required by the female for egg placement in the near future. It is possible that in these trials two nests may not have provided a high enough nest density to elicit a representative response for the effects of nest availability.

Egg damage patterns of captive cowbirds may not be a reliable tool for distinguishing between the proposed mechanisms for egg attacks because a number of stresses which should normally limit cowbird tendency to attack eggs were absent (e.g. need to guard mates, defend home ranges, locate food, and bypass host nest defense). However, despite having unlimited access to nests and eggs, the average number of eggs damaged by cowbirds in captivity was similar to rates reported from field studies (Mayfield 1960, McGeen and McGeen 1968, Elliott 1978, Nolan 1978, Wolf 1987). This suggests that there is strong selection against indiscriminate egg attacks.

The number of eggs damaged during female egg attacks appeared to be influenced by clutch size rather than the total number of eggs available. This provides additional support for the view that female egg attacks are a specific breeding strategy. Unfortunately, data from this study are inadequate for distinguishing further between the four proposed functions. Male cowbirds tended to damage a specific number of eggs, independent of clutch size or the number of nests presented. This pattern is consistent with the predictions of the predation hypothesis. However, since the hosts' response to nests with eggs could be influenced by the number of damaged eggs present rather than by the number of undamaged eggs, the breeding hypothesis cannot be excluded.

The failure of egg type to influence cowbird responses to eggs could indicate that cowbirds do not discriminate among hosts on the basis of host eggs. However, the increased tendency of cowbirds to remove blackbird eggs, rather than attack and leave them in nests, suggests that cowbirds may recognise differences in egg types. Alternatively, cowbirds may discriminate among hosts, but on the basis of adults, rather than eggs. Finally, discrimination among hosts may be limited to periods when nests are abundant and choices are available.

In summary, the results from this chapter support the hypothesis that egg removal and/or damage by cowbirds is a specific reproductive strategy and suggest that, although egg contents may be consumed, eggs are neither a major nor regular part of the cowbird's diet. Cowbirds produce approximately 40 eggs in a given breeding season (Scott and Ankney 1980). Finding appropriate host nests for egg deposition is likely, therefore, to be one of the primary factors which limit the cowbird's reproductive success. Indiscriminate predation should interfere with brood parasitism because it would decrease the availability of nests suitable for parasitic egg laying as well as increasing the chances of destruction of one's own eggs. Hence, there should be strong selection against indiscriminate egg attacks for the purpose of predation.

Of the four functions proposed for egg attacks as a specific breeding strategy, the promotion of asynchrony among hosts appears to be of the least importance. The relative importance of these functions is likely to vary, however, depending on circumstances. Because eggs are incubated by contact with the parent's brood patch (Payne 1977), clutch reduction should increase the chances that cowbird eggs will be sufficiently incubated. The opportunity to attack eggs may be limited, however, by nest accessibility (i.e. host defence) and the host nesting stage (i.e. eggs are attacked if more than one egg is present in the nest; Hann 1941, Friedmann 1963). Similarly, although the removal and/or damage of eggs might reduce competition among nestlings for parental care, the need to manipulate eggs may conflict with the need to minimize the disturbance to hosts nesting-- as the latter might lead to desertion by hosts. Results from a number of studies have indicated that the timing of cowbird egg placement, relative to host egg laying, greatly influences cowbird egg success in host nests (Norris 1947, McGeen 1971, Clark and Robertson 1981, Burgham 1985). Nonetheless, many cowbird eggs are placed in host nests outside host egg laying (i.e. before laying begins, after incubation has begun, and, occassionally, in nests with fledglings) (Friedmann 1929, Hann 1941, McGeen and McGeen 1968, Klaas 1975, Wolf 1987). This suggests that although egg attacks might function to promote synchrony between cowbird and host laying, the need to place eggs in the immediate future outweighs opportunities to improve conditons for later egg laying. A similar argument could be made for egg attacks as a mechanism to extend cowbird access to host nests.

CHAPTER TWO

THE EFFECT OF EXOGENIC PROLACTIN AND TESTOSTERONE ON COWBIRD RESPONSES TO EGGS.

INTRODUCTION

The parasitic Brown-Headed Cowbird, Molothrus ater, often manipulates (i.e. removes and/or damages) eggs in host nests (Friedmann 1929, Nice 1937, Hann 1941, Norris 1947, Mayfield 1960, 1961, 1977, Friedmann 1963, Friedmann et al. 1977, Payne 1977, Clark and Robertson 1981, Blankespoor et al. 1982, Murphy 1986, Burgham and Picman unpub.). Based on information from a number of direct observations, it appears that the contents of these eggs are frequently consumed by the cowbirds. However, predation is not believed to be the primary purpose behind these cowbird egg attacks (Friedmann 1929, Nice 1937, Mayfield 1963). Results of studies described in chapter one provide support for an alternative hypothesis which proposes that egg manipulation by cowbirds is a specific reproductive strategy to improve the chances of successful brood parasitism. The results from those studies indicate that adult cowbirds attack eggs from late May to mid-July. Throughout this time the frequency of the behavior is independent of cowbird hunger level. The abrupt decline in cowbird response to eggs at the end of cowbird egg laying suggests that cowbird egg manipulation behavior may be under hormonal control.

Dufty et al. (1987) recently showed that cowbirds exhibit elevated prolactin levels throughout the breeding season, but they were unable to suggest a purpose for the presence of prolactin. Prolactin is known as the "parental" hormone due to its involvement in brood patch development and in the maintenance of incubation behavior (Eisner 1960). However, neither of these conditions is manifested by the parasitic cowbird nor can they be induced artificially (Friedmann 1929, Selander 1960, Selander and Kuich 1963). Prolactin is also known to increase the rate of food and water intake and fat build-up in many species. This is unlikely to be the primary purpose of the cowbirds' elevated prolactin levels during breeding, because the pattern of prolactin secretion does not coincide with ovarian development, which is the greatest drain on energy for females during this period (Scott and Ankney 1983, Dufty and Wingfield 1986a, Dufty et al. 1987). It is also unclear how these factors could be related to male patterns of prolactin secretion since male energy requirements during breeding are relatively low (Dufty and Wingfield 1986a). Prolactin levels appear to reflect the cowbird tendency to attack eggs (i.e. prolactin is high throughout breeding and then presumably decrease in late July). Therefore, I hypothesize that in cowbirds, prolactin assumes an alternate role and may be involved in the control of the egg manipulation behavior.

In addition, cowbird tendency to attack eggs could be influenced by testosterone, a hormone that is usually associated with aggressive behaviors such as territoriality and mate-guarding and that has been shown to have an inhibitory effect on parental behavior (see Ensor 1978 for a review). In contrast to males of most nesting species, male cowbirds display elevated levels of testosterone throughout most of the breeding season (Dufty and Wingfield 1986a). Female cowbird testosterone levels also rise in the spring, but their subsequent decline occurs more rapidly (Dufty and Wingfield 1986b).

The purpose of this chapter is: 1) to examine responses of non-breeding, captive cowbirds to nests with eggs and 2) to investigate the effects of experimental increases in exogenic prolactin and testosterone on cowbird tendency to remove and/or damage eggs.

METHODS

Subjects and General Procedure

Post-breeding studies were initiated in September 1987 with 38 Brown-headed Cowbirds (*Molothrus ater*). These birds were obtained from the captive population discussed in chapter one [12 adult females, nine adult males, six juvenile females, and seven juvenile males] and were housed as previously described (i.e. maintained in individual cages with food and water available *ad libitum*; see chapter one for details).

Cowbird responses to nests with eggs were tested once every two weeks from early September to the end of October. Behavioral trials were initiated at 17:30 hoursby placing an artificial nest containing one treated Blue-breasted Quail egg (*Coturnix chinensis*) in a wire holder inside the cages (see chapter 1 for details on nest construction and egg treatment procedures). Eggs and nests were removed after three hours and scored for damage on the basis of 1) location of the nest and egg, 2) number and size of holes in the egg, and 3) the amount and condition (i.e. yolk pecked or whole) of egg contents.

During the second part of the study, each cowbird (eight adult males and eight adult females) was injected on three occasions. Injections were made subcutaneously over the pectoral muscles and contained 0.1 ml of a) saline [control], b) prolactin [approximately 50 I.U., Ovine Luteotropic Hormone, LTH] or c) testosterone propionate [1.0 mg/ml]. The proportion of adult cowbirds that responded positively (i.e. removed and/or damaged eggs) following the saline injection was similar to that for trials when cowbirds were not injected (males X^2=0.23, d.f.=1 p>0.50, females X^2=0.23, d.f.=1, p>0.50). It was, therefore, assumed that the injection process did not interfere with cowbird responses to eggs. The prolactin was injected on November 10th, and was followed by the testosterone treatment on November 15th. Because prolactin is believed to be metabolized in as little as 30 minutes, behavioral testing was initiated one hour after injections and continued once per day for four days. As in the first part of the study, tests were initiated at 17:30 hours and nests presented to cowbirds contained a single treated quail egg. Behavior was evaluated using the criteria described in chapter one.

RESULTS

Response to eggs

In September and October, adult and juvenile cowbirds of both sexes frequently responded to experimental nests by removing and/or damaging the egg. The proportion of juveniles that attacked was slightly, but not significantly, higher than for adults (Table 13), and neither group differed from the rate of response observed in chapter one for breeding adults (for juveniles: $X^2=0$, $p>0.9$; for adults: $X^2=1.58$, $p>0.1$). In all three cases, females tended to respond slightly, but not significantly, more often than males (for juveniles: $X^2=.73$, $p>0.25$; for post-breeding adults: $X^2=.63$, $p>0.25$; for breeding adults: $X^2=.83$, $p>0.25$). However, when the results for the three groups were combined, the difference between males and females became significant ($X^2=4.35$, $p<0.05$).

During attacks on eggs, juveniles tended to remove eggs, rather than leave damaged eggs in the nests, more often than adults (Table 13, 14). The rate of removal did not differ significantly from that reported in chapter one for trials initiated late in the breeding season for either sex, whether they be adults or juveniles (Table 14).

Effects of exogenic hormones

Prolactin

One hour after the cowbirds were injected with prolactin, the number of females that responded positively to nests had dropped from 50% to zero per cent ($X^2=5.33$, d.f.=1 $p<0.05$). None of the four females that had been attacking eggs prior to treatment pecked or removed eggs during the first three post-injection tests and only one out of the four resumed pecking during the four days of post-treatment behavioral testing (Table 15). Individuals who were unresponsive prior to the injection of prolactin did not begin pecking during the four days of post-treatment behavioral testing.

Male cowbird responses to eggs dropped slightly, but not significantly, after prolactin injections (Table 15). Of the four individuals that had been pecking, three responded on day 1 [$X^2=0.25$, d.f.=1, $p>0.5$], two on day 2 [$X^2=1.00$; d.f.=1, $p>0.25$], and then three again on days 3 and 4 [$X^2=0.25$, d.f.=1, $p>0.5$]. None of the five initially unresponsive males removed or damaged eggs during the four days of behavioral testing following the prolactin injection. Exogenic prolactin appears, therefore, to inhibit female but not male egg attacks. This contradicts the proposed hypothesis that prolactin should stimulate cowbird egg attacks.

Testosterone

The proportion of females that responded to nests with eggs gradually increased during the four days following the testosterone injection (Table 16). The difference in the frequency of responses was significant on day four by which time eight out of nine females responded positively ($X^2=5.84$, $p<0.05$). In contrast, male responses to nests with eggs did not increase significantly during the four days of behavioral testing that followed the testosterone injection (Table 16). Nor did the proportion of individuals that attacked eggs differ significantly from that of the control during any given test (for days 1 and 2:$X^2=0.25$, $p>0.5$; for day 3:$X^2=0.04$, $p>0.75$; and for day 4:$X^2=1.73$, $p>0.1$). Hence, testosterone appears to induce egg attacks in female, but not male cowbirds.

DISCUSSION

Post-Breeding Responses to Eggs

When tested in September and October, adult and juvenile cowbird responses to experimental nests with eggs were similar to those observed during the breeding period. Since the circulating levels of most hormones associated with reproduction decline at the end of breeding, the occurrence of egg attacks throughout the two months of post-breeding testing suggests that elevated hormonal levels may not be necessary to promote this behavior. Hence, if hormones do regulate cowbird responses to eggs, then the decline in adult responses observed at the end of cowbird egg laying is probably caused by some other factor.

Although sexually immature, juveniles exhibit elevated levels of prolactin throughout June and July (Dufty and Wingfield 1986 a,b). Their lack of response to eggs during this period (chapter one), suggests that prolactin may be involved in the inhibition of cowbird attacks on eggs. This view is supported by the observed effects of experimentally increased prolactin levels on cowbird responses to eggs (see discussion below).

Exogenic Hormones: Prolactin

The proportion of female cowbirds that attacked eggs decreased significantly within one hour after injections of prolactin. This argues against the hypothesis that prolactin promotes egg removal and/or damage by cowbirds. Circulating levels of prolactin are elevated throughout the breeding season when cowbird tendency to remove and/or damage eggs is high (Dufty et al. 1987). Thus, if prolactin is involved in the decrease in response to eggs observed at the end of cowbird egg laying, then either the circulating levels of this hormone should increase in late July or else the cowbird threshold for this hormone should decrease. The proportion of attacked eggs that were consumed by the cowbirds did not increase during the prolactin trials despite prolactin's role in premigritory fat build up (Eisner 1960).

It is not presently known what factors influence prolactin secretion in cowbirds (Dufty et al. 1987). A dramatic surge in prolactin is stimulated in many nonparasitic species upon exposure to nests and eggs (El Halawani et al. 1980, Goldsmith and Nicholls 1984, and Dawson and Goldsmith 1985). Although a similar response by cowbirds could explain why prolactin levels remain high throughout cowbird egg laying, it is unlikely that it would promote an increase in prolactin at the end of breeding when cowbird interest in nests has declined. If prolactin levels do not increase at the end of egg laying, the change in response could reflect a decline in cowbird threshold for prolactin, associated perhaps with the onset of photorefraction. Alternatively, a second compound could be involved which either masks the effects of prolactin during cowbird egg laying or enhances prolactin effects at the end of egg laying (i.e. synergism).

Although male cowbirds display a cyclic pattern in their responses to eggs similar to that exhibited by females, the artificial increases in prolactin did not inhibit male attacks on eggs. This difference in the effect of prolactin could be due to males having a higher threshold level for prolactin. However, since similar studies have shown that the dosage injected provides approximately two hundred times the amount of prolactin normally found in the pituitary gland (Hohn 1962), this seems unlikely. Alternatively, if the change in the effects of prolactin in female cowbirds is mediated by a second compound, then the failure of exogenic prolactin to suppress male attacks on eggs could suggest that the post-breeding circulating level of this hypothetical compound was different in males and females. Unfortunately, results of this study do not allow the further examination of this hypothesis.

It is generally believed that cowbirds are insensitive to the effects of prolactin because they do not develop brood patches or incubate eggs, despite having prolactin levels comparable to species which do manifest these conditions (Hohn 1962, Selander and

Kuich 1963, Dufty et al. 1987). Artificial increases in prolactin, similar to the one engineered in this study, are also ineffective in promoting brood patch development or incubation (Selander and Kuich 1963). Since the artificial increase of prolactin in this study decreased female cowbird response to eggs, the prolactin target tissues associated with cowbird egg attacks are likely to be different from those responsible for manifesting parental care behavior.

Circulating levels of prolactin are thought to be controlled by a negative feedback mechanism in which elevated serum levels inhibit secretion of prolactin by the pituitary gland, resulting in a subsequent decline in circulating prolactin (Phillips et al. 1985). This feedback mechanism may have influenced the results obtained by other studies which examined the effects of exogenic prolactin. However, the mechanism is unlikely to have affected the results of my study because the change in female cowbird responses to eggs occurred within one hour of the prolactin injection and was maintained for more than four days.

Testosterone

After having received an injection of testosterone, the number of females that attacked eggs gradually increased until, by the third day after threatment, significantly more females were responding positively. In contrast, testosterone had no significant effect on the number of male cowbirds that removed and/or damaged eggs. Testosterone levels exhibited by males during the breeding season greatly exceed those of females: male plasma testosterone levels range from almost 1300 pg/ml to below 300 pg/ml, female levels range from about 230 pg/ml to 160 pg/ml (Dufty and Wingfield 1986a, 1986b). It is possible, therefore, that testosterone does promote attacks on eggs in both sexes but that the artificial increase engineered in the study was not high enough to evoke a response in males. Since approximately one half of male and female cowbirds were already attacking eggs prior to injections, it is unlikely that testosterone is solely responsible for cowbird attacks on eggs.

In summary, the pattern of female cowbird egg attacks indicates that prolactin inhibits the tendency to manipulate eggs while testosterone promotes this behavior. The secretion cycles of testosterone and prolactin further suggest that, during cowbird egg laying, testosterone may induce attacks on eggs, despite elevated prolactin levels. The inhibitory effects of prolactin would become apparent at the end of breeding when testosterone levels are low, coinciding with the observed decline in cowbird responses to eggs. The failure of exogenic hormones to affect male egg attacks could be a consequence of dosage or could indicate that the physiological control of egg manipulation behavior is more complex than results with females seem to indicate.

GENERAL DISCUSSION

The results of this study suggest that cowbird tendency to remove and/or damage eggs in host nests is a specific reproductive strategy and not a form of predation. In chapter one, I investigated the effects of a number of factors on cowbird responses to eggs. The pattern of responses indicated that:

1. Cowbirds do not usually consume the contents of attacked eggs.
2. The length of food deprivation period has no effect on the frequency of attacks or the proportion of attacked eggs that are consumed.
3. Cowbird responses to eggs are not consistent throughout the spring and summer, but rather decline at the end of cowbird egg laying (end of July).
4. Attacks on eggs appear to be restricted to breeding individuals;
5. Cowbird responses to eggs are positively correlated with clutch size.
6. Cowbird responses to eggs do not seem to be influenced by the number of nests present.
7. The type and extent of damage done to eggs by females appears to be influenced by clutch size but seems to be independent of the number of nests and the overall abundance of eggs. In contrast, the extent of damage

inflicted by males appears to be affected by the absolute number of eggs present.

8. Egg attacks seem to be inhibited if some damaged eggs are present in a clutch.
9. Size, color, and host status (acceptor versus rejector) do not appear to affect cowbird response to eggs.

Findings one through six confirm the predictions of the breeding strategy hypothesis and are inconsistent with predictions of the predation hypothesis. The seventh, egg damage patterns, provides weak support for both the predation hypothesis (i.e. males damage a finite number of eggs, independent of the overall abundance of eggs) and the breeding strategy hypothesis (i.e. female damage to eggs is influenced by clutch completeness rather than the total number of eggs or nests available). The inhibition of attacks on damaged eggs (finding eight), is consistent with the breeding strategy hypothesis but not the predation hypothesis. Finding nine (effect of egg type) supports the predation hypothesis (i.e. all eggs attacked equally) but does not provide strong evidence against the breeding strategy hypothesis. The latter would require that cowbirds be able to discriminate between host eggs, and this has not been adequately demonstrated.

Four functions were proposed for egg attacks as a breeding strategy. Through this behavior cowbirds could: A) create space in host nests for their own egg(s); B) reduce competition among nestlings for parental care; C) force renesting by hosts when nests are discovered too late for successful placement of cowbird eggs; and/or D) induce renesting by hosts to promote nesting asynchrony among hosts and thus extend the period when suitable foster nests are available to cowbirds. The finding that clutch size influenced the proporton of birds which responded, while availability of nests did not, is consistent with functions A, B, and C but not D. However, captivity may have affected the relative importance of the four functions (i.e. the promotion of asynchrony among hosts may play an important role when nest densities are high).

Although the restriction of attacks on eggs to the breeding season was predicted (see Table 1), it was assumed that egg attacks would stop because cowbirds would cease to search for nests at the end of their own egg laying. The abrupt decline in attacks under captive conditions, when eggs and nests were abundant, coupled with the apparent restriction of the behavior to the breeding individuals, suggests that cowbird responses to eggs might be controlled hormonally. This view is further supported by the findings that 1) cowbirds of both sexes and all ages resumed egg attacks towards the end of molt, and 2) experimental manipulations of prolactin and testosterone influenced female tendency to attack eggs. Contrary to the hypothesis initially proposed, the addition of prolactin appeared to inhibit female egg attacks. The occurrence of elevated circulating levels of prolactin during breeding when cowbirds generally attack eggs therefore suggest that either the cowbird threshold for this hormone decreases at the end of laying, or that the prolactin threshold remains the same and a second compound is involved which either masks the effects of prolactin during cowbird egg laying or increases the effects of prolactin through synergism at the end of breeding. The lack of egg attacks by juveniles during June and July, when their prolactin levels are elevated (Dufty et al. 1987), supports the idea that prolactin may inhibit attacks on eggs.

Although approximately fifty per cent of all post-breeding cowbirds attacked eggs, regardless of their age or sex, the addition of exogenic testosterone promoted egg attacks in females only. This suggests that elevated levels of testosterone are unnecessary to promote egg attacks. The influence of exogenic testosterone on female egg attack behavior might then reflect the role of testosterone during the breeding season (i.e. testosterone may act to promote attacks while prolactin level are high).

The lack of effect of either prolactin or testosterone on male cowbird tendency to remove and/or damage eggs seems inconsistent with the hypothesis that the egg attack behavior is controlled hormonally. However, as with females, male responses to eggs appear to be influenced by the individual's breeding condition. The differences between the two sexes may, therefore, reflect different dosage requirements or may indicate that the mechanism is more complex than implied by the results for females.

Captive conditions may have affected cowbird responses to nests with eggs in a number of ways. Because females did not lay while in captivity, the need to find nests in which to place their own eggs was not an immediate concern. Accordingly, female tendency to disrupt host nesting attempts may have been greater than under normal circumstances. Damage done to eggs under captive conditions may exceed that for natural nests since host activities would presumably limit cowbird access to nests and the amount of time that could be spent damaging eggs. Similarly, the increase in cowbird tendency to leave attacked eggs in nests that was observed during the experimental trials could have led to an increase in egg damage. The priority given to egg attacks may have been higher than under normal circumstances since the time and energy budgets of captive cowbirds were drastically altered (i.e. mate guarding, defense of home ranges, need to locate food were all eliminated). Alternatively, because no hosts were associated with the experimental nests, these nests may have represented a relatively weak stimulus and possibly elicited a weaker response.

Although the effects of captive conditions must be considered, the patterns of egg attacks observed within and between years suggest that the results obtained in this study are probably a reliable reflection of the tendency of wild cowbirds to remove and/or damage eggs in host nests. These results provide firm evidence for egg attacks as a breeding strategy. The cowbirds' ability to 'customize' host nests could explain why cowbirds appear to place their eggs indiscriminately among sympatric species. Additional studies are required, however, to determine to what extent egg attacks increase cowbird brood parasitic success and to establish whether some eggs are more likely to be attacked than others.

Table 1. Summary of predictions of the two hypotheses proposed to explain the occurrence of cowbird egg attacks. 1) Predation hypothesis: eggs act as an alternative food source; and 2) Breeding Strategy hypothesis: egg attacks increase the chances of successful parasitism by A) creating space in host nests for cowbird eggs; B) reducing competition among nestlings for parental care; C) forcing renesting by hosts when nests are located too late in host breeding for successful placement of cowbird eggs; and D) inducing renesting to promote asynchrony among hosts to extend the period when host nests are available to cowbirds.

Factor	Predicted Effects	
	PREDATION HYPOTHESIS	REPRODUCTIVE STRATEGY HYPOTHESIS
1. Consumption of Egg Contents	Egg contents typically consumed	A,B,C,D: Consumption is not mandatory
2. Duration of Food Deprivation	Increases tendency to attack and consume eggs	A,B,C,D: No effect
3. Time In Season	Tendency to attack eggs should always be present	A,B,C,D: Attacks limited to cowbird breeding season

Table 1. (continued)

Factor	Predicted Effects PREDATION HYPOTHESIS	REPRODUCTIVE STRATEGY HYPOTHESIS
4. Age and Sex of Individuals who Attack Eggs	All individuals, regardless of age or sex	A,B,C,D: Restricted to breeding individuals
5. Clutch Size	No effect, all nests attacked equally	A,B,C: Increases tendency to attack D: No effect
6. Number of Nests	No effect	A,B,C: No effect D: Promotes egg attacks
7. Egg Damage	Finite number damaged, corresponds to cowbird satiation level	A,B: small, finite number damaged C,D: extensive damage done, may include complete clutch destruction

Table 1. (continued)

Factor	Predicted Effects PREDATION HYPOTHESIS	 REPRODUCTIVE STRATEGY HYPOTHESIS
8. Egg Condition	No effect	A,B,C,D: If all eggs are damaged, attacks inhibited; if only some eggs damaged, attacks may continue
9. Egg Type	No effect	Acceptor species should be attacked less frequently

Table 2. The effect of time of day on cowbird tendency to attack eggs.

	% Positive Responses (Total Trials)				TEST FOR THE EFFECT OF TIME IN DAY
	05:30	09:30	13:30	17:30	
FEMALES	21 (19)	33 (24)	44 (25)	50 (20)	$X^2=4.1$, $p>0.1$
MALES	43 (16)	32 (22)	21 (24)	44 (27)	$X^2=3.8$, $p>0.25$
TEST FOR THE EFFECT OF COWBIRD SEX	$X^2=2.08$ $p>0.1$	$X^2=0.01$ $p>0.9$	$X^2=2.9$ $p>0.05$	$X^2=0.14$ $p>0.5$	

Table 3. Description of eggs used to test the effect of egg type on cowbird tendency to remove and/or damage eggs.

Species	Egg Characteristic		
	SIZE (mm)	COLOR	HOST STATUS
Red-winged Blackbird (<u>Agelaius</u> <u>phoeniceus</u>)	24 x 17	pale blue with black and brownish -black blotches blotches and spots	acceptor
Yellow-headed Blackbird (<u>Xanthocephalus</u> <u>xanthocephalus</u>)	26 x 18	very pale blue with many tiny dark speckles and blotches	rejector
Common Quail (<u>Coturnix</u> <u>coturnix</u>)	32 X 24	cream, no markings	-
Blue-breasted Quail (<u>Coturnix</u> <u>chinensis</u>)	24 X 18	cream, no markings	-

Table 4. The effect of the number of trials from initiation of testing, on cowbird tendency to leave attacked eggs in nests rather than to remove them.

	% Responses Where Eggs Were Removed (Positive Trials)	
	FEMALES	MALES
Initial 10 Trials	70 (33)	54 (35)
Later Trials (10+)	18 (22)	22 (23)
TEST FOR THE EFFECT OF CAPTIVE TESTING	$X^2=14.02$ $p<0.001$	$X^2=6.06$ $p<0.025$

Table 5. The effect of duration of food deprivation on female cowbird responses to eggs. For these trials, it was assumed that egg contents had been consumed if at least one tenth of egg contents were missing.

Treatment	% Positive Responses (Total Trials)	% Responses Where Egg Content Was Consumed (Positive Trials)
8 Hours	25 (4)	0 (1)
13 Hours	38 (8)	0 (3)
32 Hours	50 (8)	25 (4)
Control-1 (0 Hours)*	54 (11)	33 (6)
Control-2 (0 Hours)*	50 (6)	33 (3)
TEST FOR THE EFFECT OF TREATMENT		
8 Hours	$X^2=1.03$, $p>0.25$	$X^2=0.47$, $p>0.1$
13 Hours	$X^2=0.22$, $p>0.25$	$X^2=1.20$, $p>0.1$
32 Hours	$X^2=0.04$, $p>0.5$	$X^2=0.80$, $p>0.1$
TEST FOR THE EFFECT BETWEEN TREATMENTS	$X^2=0.73$, $p>0.25$	$X^2=1.14$, $p>0.1$

* The behaviorial testing for the 8 hour and 32 hour trials was initiated at 1 pm (control-1); testing for the 13 hour trials was initiated at 6 pm (control-2).

Table 6. The effect of duration of food deprivation on male cowbird responses to eggs. For these trials, it was assumed that egg contents had been consumed if at least one tenth of the egg contents were missing.

Treatment	% Positive Responses (Total Trials)	% Responses Where Egg Content Was Consumed (Positive Trials)
8 Hours	50 (4)	25 (4)
13 Hours	62 (8)	25 (8)
32 Hours	43 (7)	29 (7)
Control-1 (0 Hours)*	40 (10)	20 (10)
Control-2 (0 Hours)*	64 (11)	18 (11)
TEST FOR THE EFFECT OF TREATMENT		
8 Hours	$X^2=0.12$, $p>0.25$	$X^2=0.04$, $p>.5$
13 Hours	$X^2=0.00$, $p>0.9$	$X^2=0.13$, $p>.25$
32 Hours	$X^2=0.01$, $p>0.75$	$X^2=0.17$, $p>.25$
TEST FOR THE EFFECT BETWEEN TREATMENTS	$X^2=0.59$, $p>0.25$	$X^2=0.03$, $p>.95$

* The behavioral testing for the 8 hour and 32 hour trials was initiated at 1 pm (control-1); the testing for the 13 hour trials was initiated at 6 pm (control-2).

Table 7. The effect of time in season on cowbird tendency to attack eggs.

Date of Testing	% Positive Responses (Total Trials)		
	ADULT FEMALES	ADULT MALES	JUVENILES
May 10-15	38 (8)	50 (16)	
May 23-27	50 (16)	53 (15)	
June 8-13	56 (16)	53 (15)	10 (10)
June 24-26	50 (16)	47 (17)	7 (13)
July 7-11	53 (15)	50 (16)	9 (11)
July 20-24	47 (15)	50 (16)	9 (11)
Aug. 4-7	6 (16)	12 (16)	

NOTE: Test for the difference in cowbird responses to eggs for trials performed during cowbird egg laying (May 10 to July 24) and outside of cowbird egg laying (Aug. 4-7).

For adult females: $X^2=10.53$, d.f.=1, $p<0.001$

For adult males: $X^2= 5.57$, d.f.=1, $p<0.01$

Table 8. The effect of clutch size and number of nests on cowbird responses to eggs.

Number of Eggs/Nest	% Positive Responses (Total Trials) FEMALES	MALES
SINGLE NEST TRIALS		
1 Egg	50 (14)	60 (15)
3 Eggs	56 (16)	54 (11)
5 Eggs	94 (16)	93 (15)
TWO NEST TRIALS		
1 Egg	40 (15)	53 (15)
3 Eggs	67 (15)	75 (15)
5 Eggs	100 (8)	88 (8)
TEST FOR THE EFFECT OF CLUTCH SIZE		
1 NEST TRIALS	$X^2=7.89$, $p<0.01$	$X^2=5.93$, $p<0.05$
2 NEST TRIALS	$X^2=8.20$, $p<0.01$	$X^2=3.93$, $p>0.05$
TEST FOR THE EFFECT OF 1 VERSUS 2 NESTS		
1 EGG CLUTCH TRIALS	$X^2=0.29$, $p>0.25$	$X^2=0.14$, $p>0.25$
3 EGG CLUTCH TRIALS	$X^2=0.35$, $p>0.25$	$X^2=1.93$, $p>0.10$
5 EGG CLUTCH TRIALS	$X^2=0.52$, $p>0.01$	$X^2=0.22$, $p>0.25$

Table 9. The effect of the total number of eggs, the number of eggs per nest (i.e. clutch size), and the number of nests available on the average number of eggs damaged by female cowbirds. The significance of the differences in responses was analyzed with either the Mann-Whitney U-test for differences between 2 means or the Kruskal-Wallis analysis of variance for differences between 3 means.

Mean Number of Eggs Damaged (±S.D.)

NUMBER OF EGGS/NEST	PER POSITIVE RESPONSE[a]	PER ATTACKED NEST[b]
<u>SINGLE NEST TRIALS</u>		
3 Eggs	1.9 (±.93)	1.9 (±.93)
5 Eggs	2.5 (±1.5)	2.5 (±1.5)
<u>TWO NESTS TRIALS</u>		
1 Egg	1.7 (±0.52)	-
3 Eggs	3.0 (±1.8)	1.9 (±0.96)
5 Eggs	5.6 (±1.7)	2.8 (±0.97)

[a] TEST FOR THE EFFECT OF THE TOTAL NUMBER OF EGGS AVAILABLE

1 NEST TRIALS $p=0.04$

2 NEST TRIALS $p=0.0037$

[b] TEST FOR THE EFFECT CLUTCH SIZE

1 NEST TRIALS $p=0.04$

2 NEST TRIALS $p=0.022$

TEST FOR THE EFFECT OF THE NUMBER OF NESTS AVAILABLE

3 EGG TRIALS $p>0.05$

5 EGG TRIALS $P>0.05$

Table 10. The effect of the total number of eggs, the number of eggs per nest (i.e. clutch size), and the number of nests available on the average number of eggs damaged by male cowbirds. The significance of the differences in responses was analyzed with either the Mann-Whitney U-test for differences between 2 means or the Kruskal-Wallis analysis of variance for differences between 3 means.

	Mean Number of Eggs Damaged (±S.D.)	
NUMBER OF EGGS/NEST	PER POSITIVE RESPONSE[a]	PER ATTACKED NEST[b]
SINGLE NEST		
3 Eggs	1.8 (0.75)	1.8 (0.75)
5 Eggs	3.4 (1.5)	3.4 (1.5)
TWO NESTS		
1 Egg	1.6 (0.35)	-
3 Eggs	3.6 (1.7)	2.1 (0.83)
5 Eggs	3.6 (2.4)	2.1 (1.2)

[a] TEST FOR THE EFFECT OF THE TOTAL NUMBER OF EGGS AVAILABLE

1 NEST TRIALS $p=0.034$

2 NEST TRIALS $p=0.022$

[b] TEST FOR THE EFFECT OF CLUTCH SIZE

1 NEST TRIALS $p=0.034$

2 NEST TRIALS $p=0.88$

TEST FOR THE EFFECT OF THE NUMBER OF NESTS AVAILABLE

3 EGG TRIALS $p>0.05$

5 EGG TRIALS $p<0.05$

Table 11. The effect of egg condition on cowbird responses to eggs.

Treatment	% Positive Response (Total Trials)	
	FEMALES	MALES
1 EGG CLUTCH		
1 Damaged Egg	9 (11)	13 (15)
Control (1 Undamaged Egg)	50 (14)	60 (15)
5 EGG CLUTCH		
5 Damaged Eggs	60 (5)	67 (3)
3 Damaged Eggs	57 (7)	50 (6)
1 Damaged Egg	75 (4)	67 (6)
Control (5 Undamaged Eggs)	94 (16)	88 (16)
TEST FOR THE EFFECT 1 DAMAGED EGG IN 1 EGG CLUTCHES	$X^2=4.74$ $p<0.05$	$X^2=8.89$ $p<0.005$
TEST FOR EFFECT OF AT LEAST 1 DAMAGED EGG IN 5 EGG CLUTCHES (d.f.= 1)	$X^2=4.57$, $p<0.05$	$X^2=3.06$, $p<0.10$
TEST FOR THE EFFECT OF 1, 3, or 5 DAMAGED EGGS IN 5 EGG CLUTCHES (d.f.= 2)	$X^2=0.37$ $p>0.9$	$X^2=0.42$ $p>0.9$

Table 12. The effect of egg type on cowbird tendency to attack eggs.

EGG TYPE*		% POSITIVE RESPONSE (Total Trials)	TEST FOR THE EFFECT OF EGG TYPE
HOST STATUS	Acceptor Species	50 (14)	$X^2=0.74$, $p>0.25$
	Rejector Species	67 (12)	
EGG SIZE	Large	48 (33)	$X^2=0.1$, $p>0.1$
	Small	44 (16)	
EGG COLOR	Creamy	48 (33)	$X^2=0.01$, $p>0.5$
	Pale blue with dark markings	50 (14)	

* See Table 3 for details on egg types.

Table 13. Cowbird post-breeding responses to nests with eggs.

	% Positive Responses (Total Trials)	% Responses Where Eggs Were Removed (Positive Trials)
ADULT FEMALES	49 (45)	9 (22)
ADULT MALES	40 (35)	21 (14)
JUVENILE FEMALES	67 (15)	40 (10)
JUVENILE MALES	52 (21)	36 (11)
TEST FOR THE EFFECT OF COWBIRD SEX		
ADULTS	X^2=0.63, $p>0.25$	X^2=1.09, $p>0.25$
JUVENILES	X^2=0.73, $p>0.25$	X^2=0.03, $p>0.75$
TEST FOR THE EFFECT OF COWBIRD AGE	X^2=0.46, $p>0.25$	X^2=4.41, $p<0.05$

Table 14. The effect of breeding condition and age on cowbird tendency to leave attacked eggs in nests rather than remove them.

	% Responses Where Eggs Were Removed (Positive Trials)	
	FEMALES	MALES
POST-BREEDING ADULTS	9 (21)	21 (14)
POST-BREEDING JUVENILES	40 (10)	36 (11)
CONTROL (BREEDING ADULTS)	18 (22)	22 (23)
TEST FOR THE EFFECT OF AGE (POST-BREEDING TRIALS)	$X^2=4.31$ $p<0.05$	$X^2=0.68$ $p>0.25$
TEST FOR THE EFFECT OF BREEDING CONDITION		
ADULTS	$X^2=0.77$ $p>0.25$	$X^2=0.25$ $p>0.5$
JUVENILES	$X^2=1.75$ $p>0.1$	$X^2=0.82$ $p>0.25$

Table 15. The effect of exogenic prolactin on cowbird tendency to attack eggs (hormones were injected on Day 1, one hour prior to behavioural testing).

Treatment	% Positive Responses					TEST FOR THE EFFECT OF TREATMENT
	PRE-TEST	DAY 1	DAY 2	DAY 3	DAY 4	
<u>FEMALES</u>						
Control	44	33	33	44	33	$X^2=0.57$, $p>0.95$ (d.f.=4)
Prolactin	50	0	12	0	12	$X^2=10.6$, $p<0.05$ (d.f.=4)
<u>MALES</u>						
Control	44	33	44	44	33	$X^2=0.56$, $p>0.95$ (d.f.=4)
Prolactin	44	33	22	33	33	$X^2=1$, $p>0.9$ (d.f.=4)

Table 16. The effect of exogenic testosterone on cowbird tendency to attack eggs (hormones were injected on Day 1, one hour prior to behavioral testing).

Treatment	% Positive Responses PRE-TEST	DAY 1	DAY 2	DAY 3	DAY 4	TEST FOR THE EFFECT OF TREATMENT
FEMALES						
Control	44	33	33	44	33	$X^2=0.57$, $p>0.95$ (d.f.=4)
Testosterone	33	33	67	78	89	$X^2=9.81$, $p<0.05$ (d.f.=4)
MALES						
Control	44	33	44	44	33	$X^2=0.56$, $p>0.95$ (d.f.=4)
Testosterone	33	44	44	33	71	$X^2=2.97$, $p>0.5$ (d.f.=4)