Acceptance or Rejection of Cowbird Parasitism: Cues Used in Decision-Making by Yellow Warblers (*Dendroica petechia*)

by

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ABSTRACT

The proximate causes triggering nest abandonment are unclear for most species, including the Yellow Warbler (*Dendroica petechia*), which abandons nests parasitized by cowbirds (via burial or desertion). Cowbird parasitism and rejection of parasitism are costly to some hosts; therefore cues affecting their responses have important evolutionary implications. Manipulative experiments showed that experimentally adding a cowbird egg elicited similar rejection frequencies (2008: 31.8%; 2009: 26.1%) as naturally laid cowbird eggs (2008: 27.1%; 2009: 20.0%). In 2008, interaction with an egg-removing model increased the probability of abandonment and the most aggressive individuals were more likely to bury the model cowbird egg. In 2009, eggs added to nests before sunrise were rejected at a frequency (29.7%) similar to eggs added to nests after sunrise (22.9%). Yellow Warblers returning to nests after egg addition peered significantly longer at their clutch than at control nests, shuffled their bodies more frequently when on the eggs and spent more time probing eggs with their bill once settled on their parasitized clutch. Furthermore, although non-mimetic blue eggs were not abandoned significantly more frequently than cowbird eggs (blue 31.1% versus cowbird 21.4%), only blue eggs were ejected from some nests. Thus, warblers use both tactile and visual cues to detect the presence of a parasitic egg in their nest. Eggs added to nests were not rejected at a lower frequency than naturally parasitized nests, as was recorded in a previous study. It is difficult to know if this increase in abandonment of experimental eggs is due to phenotypic plasticity, genetic changes, or other factors. Egg recognition abilities may have changed because I have shown that the warblers' behaviour changes before versus after egg addition, whereas no changes were recorded in an earlier study. Finally, not all

individuals that buried eggs for the first time in 2009 (21.4%) buried again after being re-parasitized (5.3%), when less time remained in the breeding season relative to the first parasitism event. This suggests that egg rejection and host responsiveness in warblers, and likely other avian hosts that use abandonment as a form of rejection, is affected by environmental cues which may act as genetic expressers.

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GENERAL INTRODUCTION

Nest abandonment and decision-making

Nest abandonment occurs in diverse groups of animals, such as insects (Zink 2003), fish (Kynard, 1978, Coleman *et al.* 1985), and birds (Hill and Sealy 1994, Hosoi and Rothstein 2000). Parents may abandon their nest and its site (nest desertion) or just their nest (burial) at any stage: pre-egg laying, laying, or incubation. According to parental investment theory (Trivers 1972), parents decide to abandon if the benefits of staying with their current nest are outweighed by the potential benefits gained by abandonment (Coleman *et al.* 1985, Winkler 1991).

Early studies of parental investment focused predominately on determining whether or not animals commit the "Concorde fallacy"; decisions that are not based on the expected benefits of current offspring, but rather on preventing the loss of accumulated investment (Weatherhead 1979, Dawkins and Brockman 1980). This much-debated issue was soon resolved. Researchers noted that past investment also affects the relative value of current offspring if life-history theory is incorporated into parental investment theory (Coleman and Gross 1991, Ackerman and Eadie 2003). A parent's ability to reproduce in the future may be influenced by past investment because reproduction imposes costs on the parent. Parents may not be acting fallaciously if the value of current offspring relative to prospects for future reproduction is determined by assessing past investment (Coleman and Gross 1991).

Recently, researchers have studied how animals adjust their level of parental care based on the relative roles of past investment. Past investment may diminish residual reproductive value and the expected benefits from current offspring (Ackerman and Eadie

2003). Female Mallards (*Anas platyrhynchos*) adjust parental care (i.e., abandon or stay with their nest) based on expected benefits from their current offspring and not on past investment's reduction of future reproductive prospects (Ackerman and Eadie 2003). Ackerman *et al.* (2003) recorded that after partial clutch depredation dabbling ducks use multiple cues (proportion of clutch remaining, incubation stage, and presence of eggshells) to decide whether to abandon or stay with their nests. Finally, the number of eggs lost strongly influences whether nests are abandoned by Northern Shovelers (*A. clypeata*) and Blue-winged Teals (*A. discors*) (Armstrong and Robertson 1988).

The decision-making process, which is not restricted to parental investment, can be well explained using evolutionary psychology (Schaller *et al.* 2007). Recent research on reasoning in humans has found that information, which is retained in working memory, is manipulated and integrated. Information enters working memory in two ways: it is perceived directly from the external environment or it results from learned associations that were archived in long-term memory (Schaller *et al.* 2007). It is adaptive for human and non-human animals to selectively access the precise archived information pertinent to problems of the individual's social life. This information should readily lead to fitness-optimizing solutions to these problems (Schaller *et al.* 2007). Stimulus-response algorithms are simple evolved cognitive algorithms in which cues act as a stimulus and automatically activate specific cognitive constructs in working memory, which disposes individuals to respond in ways that benefit their fitness (Schaller *et al.* 2007).

Cues that elicit nest abandonment by non-human animals, however, remain unclear in most cases. Clutch or brood reduction seems to elicit abandonment in insects

(Zink 2003), fish (Jennions and Polakow 2001), and birds (Armstrong and Robertson 1988, Sealy 1992, Hill and Sealy 1994, Kosciuch *et al.* 2006), but not always, especially in birds (Peer *et al.* 2005). In birds, nests may also be abandoned in response to brood parasitism, but the stimulus for abandonment apparently involves more than the mere presence of the parasitic egg in the nest (Graham 1988, Hill and Sealy 1994).

Costs of avian brood parasitism and rejection of a parasitized clutch

Avian brood parasites, such as cowbirds and cuckoos, lay their eggs in nests of other species of birds, the hosts, which raise the parasite's young (Davies 2000). Once a cowbird egg is laid, some costs imposed on the host include: egg breakage due to the stronger parasitic eggs breaking the weaker host eggs during or after parasitism; inefficient incubation and/or the parasite hatching earlier than the hosts, thus decreasing hatching success of the host eggs; and parasites crowding and outcompeting the host nestlings for parental care and therefore, decreasing nestling survival (Lorenzana and Sealy 1999). Female cowbirds may also remove a host egg before or after parasitism (Sealy 1992). Finally, some adult hosts may feed fledged cowbirds over their own young (Rasmussen and Sealy 2006). Natural selection should favour the development of defences against brood parasitism because of the costs imposed on the hosts (Rothstein 1975a, Lorenzana and Sealy 1999).

Hosts may eject the egg from their clutch or abandon the parasitized clutch to reject the parasitic egg and reduce fitness costs (Rothstein 1990). Egg discrimination is well developed in many cuckoo hosts, which have had a long co-evolutionary history with the parasite (Davies and Brooke 1998). Ejection of non-mimetic eggs is well documented in these hosts, with many ejecting more than 80% of parasitic eggs (Davies

and Brooke 1998). However, only about 10% of cowbird hosts regularly eject non-mimetic cowbird eggs (Rothstein 1982a, Peer and Sealy 2004). Ejection is widely considered an anti-parasite adaptation stimulated by the presence of a parasitic egg in the nest (Rothstein 1975a, Sealy and Bazin 1995, Lorenzana and Sealy 2001) and it is well known that visual cues facilitate egg ejection by hosts of avian brood parasites (Rothstein 1975a, 1982; Underwood and Sealy 2006a, 2006b). Abandonment usually requires other cues that are still unknown within and outside the context of brood parasitism (Rothstein 1975a, Hosoi and Rothstein 2000, Peer *et al.* 2005). Factors such as life-history traits and gape-size limitations may account for the prevalence of abandonment (Servedio and Hauber 2006). Species that do not grasp-eject cowbird eggs may not be able to discriminate between their own eggs and the parasite's egg(s) or their bills may be too small to grasp the egg (Sealy 1996, Peer *et al.* 2005, Underwood and Sealy 2006a, Rasmussen 2008).

Graham (1988) reported that unparasitized nests and nests of five species to which cowbird eggs had been added by researchers were abandoned much less frequently than naturally parasitized nests. He suggested that parents respond to something other than, or in addition to, the presence of the cowbird egg in the nest. Cedar Waxwings (*Bombycilla cedrorum*) abandon their nests specifically in response to cowbird eggs, though abandonment sometimes follows their failed attempts to eject the eggs with their bills (Rothstein 1976). Reductions in clutch volume associated with cowbird egg removal causes adults to abandon their nests in the Eastern Phoebe (*Sayornis phoebe*) and Clay-coloured Sparrow (*Spizella pallida*) (Rothstein 1986, Hill and Sealy 1994). Parasitized nests are abandoned by Field Sparrows (*S. pusilla*) if they observe a cowbird at their nest

(Burhans 2000). Moksnes and Røskaft (1989) reported that Meadow Pipits (*Anthus pratensis*) abandoned up to 50% of their nests only when a stuffed cuckoo was placed near the nest and when a host egg was replaced with a cuckoo egg. Few nests were abandoned if a stuffed cuckoo was placed near the nest without manipulating the clutch or if the clutch was manipulated without presenting the stuffed cuckoo near the nest (Moksnes and Røskaft 1989).

Desertion and burial unrelated to brood parasitism

Abandonment via desertion may be a response to events unrelated to brood parasitism. Male Penduline Tits (*Remiz pendulinus*) build nests that are then chosen by the females (Hoi *et al.* 1994). The larger nests ensure ideal thermal conditions, but smaller nests are abandoned, usually after the females have built the bottom layer of the nest and laid one or two eggs (Hoi *et al.* 1994). Visits by humans or other predators at the nest may cause clutch abandonment (Rothstein 1975a; Guigueno and Sealy, in press) and species not exposed to brood parasitism may desert nests after partial clutch loss (Armstrong and Robertson 1988, Winkler 1991, Delehanty and Oring 1993). Kosciuch *et al.* (2006) reported that parasitized Bell's Vireos (*Vireo bellii*) respond specifically to partial or complete clutch loss by deserting their nests, a behaviour that brood parasitism does not appear to have altered (also Hill and Sealy 1994). Because egg predation causes abandonment in many bird species, it is important to examine whether abandonment also is an adaptation to brood parasitism and which aspects cause desertion.

Factors other than parasitism, such as inclement weather or visits by predators may elicit burial (Rothstein 1975a). Rothstein (1975a) added artificial cowbird eggs to nests of different host species and recorded the only case of burial during "unusually

stormy weather" at an Eastern Phoebe nest. The phoebe may have buried its clutch in response to the weather and not to reject the cowbird egg (Rothstein 1975a). Burial also occurs at Yellow Warbler nests that apparently were not parasitized or experimentally parasitized with conspecific eggs, although this is rare (six out of approximately 1800 nests [Sealy 1995]). During these events, warblers buried their clutch usually following the loss of egg(s) that may have been taken by a predator or when the parent skipped a day during laying (Goossen 1985, Sealy 1995).

Are burial and desertion anti-parasite defences?

The Yellow Warbler is the only host of the Brown-headed Cowbird (*Molothrus ater*) that abandons parasitized clutches by burying cowbird eggs, often with its own eggs, then laying a new clutch in a superimposed nest (Sealy 1995, Mico 1998). Yellow Warblers also abandon their nest and nest site (nest desertion) (Sealy 1995). Rothstein (1975a, b) considered burial and desertion standard behavioural "by-products" (i.e., not anti-parasite defences) and he concluded that Yellow Warblers are accepters because every cowbird egg he experimentally added to 16 nests was accepted. However, subsequent work revealed that (1) Yellow Warblers buried ~37 % of naturally parasitized clutches versus 0% of control clutches (Sealy 1995), (2) burial usually occurred up to the midpoint of laying, but acceptance prevailed in incubation by which time the threat of parasitism had decreased (Clark and Robertson 1981, Sealy 1995) and, (3) reproductive success at unparasitized nests and at nests with buried cowbird eggs were similar (Clark and Robertson 1981). Therefore, burial is a specialized anti-parasite defence (Clark and Robertson 1981, Sealy 1995).

Nest desertion also may be an anti-parasite defence (Hosoi and Rothstein 2000, Guigueno and Sealy, in press). Brood parasitism may select for a high frequency of nest desertion in passerines, because species nesting in open habitats desert more frequently than forest-nesting species, which are less likely to encounter cowbirds (Hosoi and Rothstein 2000). Also, Yellow Warblers deserted naturally parasitized nests at a significantly higher frequency than control nests (chi-square test with continuity correction, $\chi^2 = 22.64$, df $= 1$, $p < 0.0001$; Sealy 1995). Nest desertion may evolve more readily than ejection because ejection requires egg recognition abilities, but once egg recognition abilities evolve, ejection quickly spreads through the population (Hosoi and Rothstein 2000).

A recent study examined the differences between burial and desertion at naturally parasitized nests and the circumstances surrounding each response (Guigueno and Sealy, in press). Results showed that damaged nests and clutches reduced to zero, one, or two host eggs (i.e., probably interference by a predator or inclement weather) were deserted, whereas burial occurred when zero, one, or two host eggs were present the morning cowbirds laid, when the parasitic egg had a high probability of hatching (Guigueno and Sealy, in press). Response times for burial varied less than for desertions although both behaviours were recorded on average ~2.4 days after the parasitism event. Burial was more frequent, though desertion was recorded one-third of the time. Desertion, therefore, may be elicited by more factors unrelated to brood parasitism than burial, such as interference by predators and inclement weather. Desertion is a more general response, whereas burial appears to be more specific to brood parasitism (Guigueno and Sealy, in press). However, Yellow Warblers may use both behaviours as anti-parasite defences

(Sealy 1995, Hosoi and Rothstein 2000), but they are considered intermediate rejecters because a species must accept or reject at a frequency of 80% or higher to be considered an accepter or rejecter (Rothstein 1975a, b); Yellow Warblers reject naturally parasitized nests at ~ 40% frequency (Sealy 1995).

Yellow Warblers apparently do not visually recognize their own eggs (Sealy and Lorenzana 1998) and their bills may be too small to eject cowbird eggs (Clark and Robertson 1981, Rohwer and Spaw 1988, Sealy 1995, Rasmussen 2008); therefore, abandonment may be the only rejection option available to this species. Nest desertion is likely more costly than burial because: (1) time and effort are spent finding a site for the new nest, (2) the new clutch is initiated 3-6 days later (Clark and Robertson 1981), and, (3) parasitism occurs more frequently at the new location than in the refurbished nest (Burgham and Picman 1989). Abandonment via burial or desertion, however, occurs much less frequently in response to experimental parasitism. For nests naturally parasitized on the day after clutch initiation (Laying Day 2; LD2), rejection in response to natural parasitism was much more frequent (48.6%) than experimental parasitism (8.5%) (a real cowbird egg was added to warbler nests after females laid their own eggs; Sealy 1995). The cues that elicit rejection in Yellow Warblers remain unknown (Sealy 1995, Peer *et al.* 2005).

Timing of clutch manipulation experiments on Yellow Warblers

Most studies that have tested host responses to experimentally added cowbird eggs did not adequately simulate natural parasitism. Hosts that eject cowbird eggs from their nests respond visually to the presence of the cowbird egg and experimental parasitism elicits ejection at a similar frequency to natural parasitism (Rothstein 1975a,

b). However, other hosts, such as Yellow Warblers, which likely cannot eject cowbird eggs and/or cannot recognize their own eggs rely on other cues to eliminate parasitism. Therefore, it is important to simulate natural parasitism as closely as possible when testing these hosts' responses. Although most cowbirds parasitize nests during a half-hour-to-hour "window" of time before sunrise (McMaster *et al.* 2004), no study has ever involved experimentally parasitized groups of nests consistently at that time. Rothstein (1975a) added model cowbird eggs and immediately removed a host egg from the nests of 31 host species, including the Yellow Warbler. All nests were parasitized "up to seven hours after sunrise" (Rothstein 1975a: 252). Burgham and Picman (1989) placed either real or model cowbird eggs into Yellow Warbler nests to add to the small sample of naturally parasitized nests, however, they did not specify the time of experimental parasitism. Cowbird visits were simulated by placing a caged female cowbird by the nests between 07:00 and 10:00 hrs CDT (Burgham and Picman 1989). To determine whether rejection frequency is influenced by host egg removal, Sealy (1992) added cowbird eggs to warbler nests between 07:00 and 10:00 hrs CDT without removing a host egg in one group, but switched a host egg with a cowbird egg in another group. Cowbirds, however, usually remove a host egg during a different visit to the nest (Sealy 1992). In addition to adding real cowbird eggs to nests at different nesting stages, Sealy (1995) also combined experimental parasitism and the placement of a female cowbird model in laying position on warbler nests. All of Sealy's (1995) experiments were conducted between 07:00 and 10:00 hrs CDT and he recorded the warblers' acceptance/rejection responses to parasitism. Despite presenting a laying cowbird model as well as adding a cowbird egg to

nests, Sealy (1995) recorded lower rejection frequencies than what was observed at naturally parasitized nests.

In an egg-recognition experiment on Yellow Warblers, different combinations of cowbird eggs and host eggs were presented to warblers after clutch completion. All experiments were conducted after 07:00 hrs CDT (Sealy and Lorenzana 1998). Tewksbury *et al.* (2002) recorded Yellow Warblers' incubation behaviour after they played cowbird vocalizations one meter from a nest and "later in the same day" switched a host egg with a real cowbird egg. Cowbird eggs were also added to other nests to determine the effect of parasitism on predation rates, although the time of cowbird egg addition was not indicated (Tewksbury *et al.* 2002).

Brown-headed Cowbirds lay about 31 minutes before sunrise (McMaster *et al.* 2004). Yellow Warblers lay their own eggs about 13 minutes after sunrise (McMaster *et al.* 2004), thus nests have been experimentally parasitized after the females have laid their eggs, but during natural parasitism, nests were parasitized minutes to hours before. The more experimental parasitism resembles natural parasitism, the more likely warblers will behave naturally and, perhaps, with that manipulation alone, rejection will occur with frequencies that are similar at experimental nests and naturally parasitized nests.

Effect of host egg-removal by cowbirds on rejection

Cowbirds remove one host egg from about one in three Yellow Warbler nests they parasitize (Sealy 1992), unlike predators that usually remove all eggs (Sealy 1994). Egg removal, however, does not influence the likelihood that the warbler will accept the parasitic egg (Sealy 1992). Cowbirds that ingest host eggs gain nourishment, but they could remove more eggs without eliciting nest abandonment by the host (i.e., two could

be removed from a 4-egg clutch and three from a 5-egg clutch; Sealy [1992]). It is

possible that cowbirds do not remove host eggs from all nests they parasitize because

trips to the nest increase the risk that parasites will interact with the hosts (Sealy 1992).

Davies and Brooke (1988) have shown that hosts are more likely to reject a parasitic egg

if they interact with the parasite. Common Cuckoos (*Cuculus canorus*) parasitize Reed

Warblers (*Acrocephalus scirpaceus*) in the afternoon, when these hosts are frequently

inattentive; parasitizing them in the morning would lead to rejection (Davies and Brooke

1988). Although the function of egg-removal is not known, it is likely a breeding strategy

and not a form of predation (Sealy 1992).

Cowbirds more frequently remove Yellow Warbler eggs during the host's LD2

(26.7 % LD1 or LD2, 33.3% LD2; 10.0% LD2 or LD3). This finding is consistent with

that from Nolan's (1978) work on the Prairie Warbler (*Dendroica discolour*), where it

was noted that cowbirds parasitize nests most frequently during LD2 (15 % LD1 or LD2;

31% LD2; 13% LD2 or LD 3). As cowbirds parasitize nests so early in the morning,

Sealy (1992) assumed and later determined that cowbirds rarely remove host eggs before

laying. Also, cowbirds remove host eggs most frequently after they have parasitized a

nest, as 33.3% of eggs were removed a day before or on the same day they laid, 20.8% on

the same day they laid, and 46% on the same or subsequent days (Sealy 1992). Nolan

(1978) found similar results with Prairie Warblers (22% a day before or day of laying;

28% day of laying; 7% day of laying or day after; 19% one or more days after laying).

No one has ever simulated a host egg-removal event using a female cowbird model in

combination with experimental parasitism of nests to determine rejection frequency.

Addition of non-cowbird egg objects to Yellow Warbler nests

Other eggs have been experimentally or naturally added to Yellow Warbler nests. Sealy (1989) reported an incidental "egg dumping" event by a House Wren (*Troglodytes aedon*). That wren egg was not rejected, but Sealy (1989) experimentally introduced other wren eggs into warbler nests at different nesting stages. All eggs added into unlined nests were buried ($n = 3$), likely because the warblers simply continued to build their nest, but eggs added to nests that were lined or contained eggs were generally accepted ($n = 21$). Burial was reported once in the latter group (Sealy 1989). Sealy and Lorenzana (1998) found that Yellow Warblers accepted all non-mimetic and small Least Flycatcher (*Empidonax minimus*) (white) and small blue eggs added to their nests. Finally, Yellow Warblers accepted all foreign warbler eggs added to their nests (Sealy *et al.* 1989).

Foreign objects or eggs other than cowbird eggs have elicited burial or nest desertion at high frequencies relative to cowbird eggs experimentally added to their nests. Stewart (2003) reported that 37% of 27 model Black-billed Cuckoo eggs (*Coccyzus erythropthalmus*) and 27 real American Robin eggs (*Turdus migratorius*) placed in warbler nests during early laying were buried or nests were deserted. These eggs were larger, heavier and coloured differently than cowbird and warbler eggs (Rothstein 1982a, Stewart 2003). It is unknown whether the difference in size or colour elicited abandonment. Stewart (2003) suggested that it would be necessary to add cuckoo-sized Yellow Warbler egg models to nests to determine this. A cue that may have stimulated the warblers to reject the larger cuckoo and robin eggs may have been the degree of "crowding" in their nests (Stewart 2003). During incubation, birds usually reach beneath the eggs in their nest with their bills and rotate or move the eggs (Deeming 2002). As the

model cuckoo or real robin eggs were usually found in the middle of the nest cavity, surrounded by host eggs during nest visits, it is possible that the warblers had difficulty adjusting the eggs' location in their nests (Stewart 2003).

Kuehn (2009) added two blue cowbird-sized eggs in the nests of Yellow Warblers in populations sympatric and allopatric with cowbirds and also in naïve populations (where cowbirds are locally uncommon) and reported abandonment on the second day of the experiment at 13.0% to 36.8% of nests. Naïve populations abandoned experimentally parasitized clutches less frequently than sympatric populations, suggesting that the warblers' rejection behaviour is plastic and increases with cowbird exposure.

Burial by Yellow Warblers has also been elicited frequently after non-egg-shaped objects were added to nests early in laying (Guigueno and Sealy 2009). Least egg-shaped objects (stars) were rejected (most buried) more frequently (43.8%) than egg-shaped objects (16.3%; cowbird eggs) (Guigueno and Sealy 2009). This suggests that stars may have created more disturbance in the nest, as they are less easily moved from the nest cavity. Nest sanitation (i.e., removal of fecal sacs, experimental non-egg object and similar debris from the nest) may be a pre-adaptation to rejection of parasitic eggs, especially burial, in the Yellow Warbler (Guigueno and Sealy 2009). Similarly, the Great Reed Warbler (*Acrocephalus arundinaceus*), a Common Cuckoo host that also buries and deserts parasitized clutches, rejected non-egg objects more frequently than cuckoo eggs, which led Moskát *et al.* (2003: p. 18) to conclude that nest sanitation behaviour may be "*an important evolutionary stage*" leading to the rejection of parasitic eggs.

Despite extensive research by S. G. Sealy and co-workers spanning nearly three decades, however, the cue(s) that elicit rejection in Yellow Warblers under natural

conditions have not been determined. Cowbird parasitism elicits this response (Sealy

1995), but the precise cues are still unknown.

CLUTCH ABANDONMENT IN YELLOW WARBLERS: CUES USED IN

DECISION-MAKING

Nest abandonment occurs in diverse groups of animals, such as insects (Zink 2003), fish (Kynard, 1978, Coleman *et al.* 1985), and birds (Hill and Sealy 1994, Hosoi and Rothstein 2000). According to parental investment theory (Trivers 1972), parents decide to abandon if the benefits of staying with their current nest are outweighed by the potential benefits gained by abandonment (Coleman *et al.* 1985, Winkler 1991). Cues that elicit nest abandonment, however, remain unclear in most cases (Peer *et al.* 2005). Cues act as stimuli and activate specific cognitions in working memory, which dispose individuals to respond in ways that benefit their fitness (Schaller *et al.* 2007). Clutch or brood reduction seems to elicit abandonment in insects (Zink 2003), fish (Jennions and Polakow 2001), and birds (Armstrong and Robertson 1988, Sealy 1992, Hill and Sealy 1994, Kosciuch *et al.* 2006), but not always, especially in birds (Peer *et al.* 2005). Bird nests may also be abandoned in response to brood parasitism, but the stimulus for abandonment apparently involves more than the mere presence of the parasitic egg in the nest (Graham 1988).

Avian brood parasites, such as cowbirds and cuckoos, lay their eggs in nests of other species of birds, the hosts, which raise the parasite's young (Davies 2000). Natural selection should favour the development of defences against brood parasitism because of the costs imposed on the hosts (Rothstein 1975a, Sealy 1992, Lorenzana and Sealy 1999, Rasmussen and Sealy 2006). Hosts may eject the egg from their clutch or abandon the parasitized clutch to reject the parasitic egg and reduce fitness costs (Sealy 1995, Rothstein 1990, Hosoi and Rothstein 2000). Abandonment usually requires other cues

that are largely unknown within and outside the context of brood parasitism (Rothstein 1975a, Hosoi and Rothstein 2000, Peer *et al.* 2005). Factors such as life-history traits and gape-size limitations may account for the prevalence of abandonment (Servedio and Hauber 2006). Species that do not grasp-eject cowbird eggs may not be able to discriminate between their own eggs and the parasite's egg(s) or their bills may be too small to grasp the egg (Sealy 1996, Peer *et al.* 2005, Underwood and Sealy 2006a, Rasmussen 2008). Therefore, they may rely on other cues, such as interaction with the brood parasite (Moksnes and Røskaft 1989, Sealy 1995, Hosoi and Rothstein 2000) to determine they have been parasitized.

The Yellow Warbler is the only host of the Brown-headed Cowbird that abandons parasitized clutches by burying cowbird eggs, often with its own eggs, and then lays a new clutch in a superimposed nest (Sealy 1995, Mico 1998). They also abandon their nest and nest site (nest desertion) (Sealy 1995). Burial is a specialized anti-parasite strategy because: (1) Yellow Warblers buried ~37 % of naturally parasitized clutches versus no control clutches (Sealy 1995), (2) burial usually occurred up to the midpoint of laying, but acceptance prevailed in incubation when the threat of parasitism was lower (Clark and Robertson 1981, Sealy 1995) and, (3) reproductive success at unparasitized nests and nests with buried cowbird eggs was similar (Clark and Robertson 1981).

Nest desertion also may be an anti-parasite defence (Hosoi and Rothstein 2000, Guigueno and Sealy, in press). Brood parasitism may select for a high frequency of nest desertion in passerines, because species nesting in open habitats desert more frequently than forest-nesting species, which are less likely to encounter cowbirds (Hosoi and Rothstein 2000). Also, Yellow Warblers deserted naturally parasitized nests at a

statistically higher frequency than control nests (Sealy 1995). Egg recognition abilities may be more difficult to evolve than desertion, but once evolved, ejection behaviour quickly spreads through the population (Hosoi and Rothstein 2000, Peer *et al.* 2005, Underwood and Sealy 2006b).

Although most cowbirds parasitize nests during a half-hour to hour "window" of time before sunrise (McMaster *et al.* 2004), no study examining host responses to parasitism has ever experimentally parasitized groups of nests consistently at that time (Rothstein 1975a, Burgham and Picman 1989, Sealy 1995). Yellow Warblers lay their own eggs after sunrise (McMaster *et al.* 2004), thus nests were parasitized after host egg-laying in previous clutch manipulation studies (Rothstein 1975a, Burgham and Picman 1989, Sealy 1995), but minutes to hours before during natural parasitism (McMaster *et al.* 2004). In addition to adding real cowbird eggs to nests at different nesting stages, Sealy (1995) also combined experimental parasitism and the placement of a female cowbird model in laying position on warbler nests. All of Sealy's (1995) experiments were conducted between 07:00 and 10:00 hrs and recorded the warblers' acceptance/rejection responses to parasitism. Despite presenting a laying cowbird model in combination with adding a cowbird egg to nests, Sealy (1995) did not record rejection frequencies at experimental nests (~8%) as high as was observed at naturally parasitized nests (~45%).

Yellow Warblers respond more aggressively to a cowbird in a perching position after being exposed to an egg-removing cowbird than after being exposed to a laying cowbird (Campobello 2008), suggesting that interaction with an egg-removing cowbird may be more important in eliciting rejection of a parasitized clutch. After exposure to the egg-removing model, warblers increased the number of *seet* calls, attacks on the model,

and distraction displays, however, after being exposed to the laying model, warblers only significantly increased the number of *seet* calls. Campobello (2008) concluded that a threatening experience causes warblers to react more aggressively in successive cowbird encounters. The rate of *seet* calls may indicate the female's level of aggressiveness to the model (Gill 1995, Gill and Sealy 2004). Cowbirds remove one host egg, more frequently during the host's LD2, from about one in three Yellow Warbler nests they parasitize (Sealy 1992), unlike predators that usually remove all eggs (Sealy 1994). Egg removal itself, however, does not influence the likelihood that the warbler will accept the parasitic egg (Sealy 1992).

Hypotheses

The cue(s) eliciting rejection in Yellow Warblers in natural situations have not been determined. Cowbird parasitism does elicit this response (Clark and Robertson 1981, Burghardt and Picman 1989, Sealy 1995), but the precise cues remain unknown. The objectives are to study which aspect(s) of brood parasitism (i.e., clutch manipulations, interaction with brood parasites, etc…) elicit(s) rejection. Abandonment of nests imposes reproductive costs in terms of lost time and energy (Clark and Robertson 1981). Although this study deals with cues associated with parasitism that elicit abandonment by a host, I will also gain insight into the level of disturbance a songbird can tolerate before it abandons its nest (Sealy 1992). Hypothesis-testing occurred over two field seasons, with hypotheses 1 and 2 tested in 2008 and hypotheses 3 to 5 in 2009. Results from hypotheses 1 and 2 influenced the selection of hypotheses 3 to 5.

1. Host-parasite interactions in concert with clutch manipulations – I investigated whether (1) adding a cowbird egg to nests before sunrise, (2) removing a

host egg after sunrise, (3) the presence of a laying cowbird before sunrise (simulation of laying event), and/or (4) the presence of an egg-removing cowbird after sunrise (simulation of egg-removal event) were cues for rejection. Previous work has shown that adding a cowbird egg to nests (Sealy 1995) and removing a host egg (Sealy 1992) do not elicit rejection at the same frequency as natural parasitism, therefore I had predicted that these cues alone were not likely to be the cues used in the decision to abandon, even if past experimental parasitism had not occurred during the natural time for parasitism (i.e., before sunrise and before warblers laid their own egg; Sealy 1995). Yellow Warblers have been disturbed before sunrise (Neudorf and Sealy 1994, McMaster *et al.* 2004), but human visits did not elicit abandonment (see also Sealy 1995).

I hypothesized that interactions with cowbirds during acts of laying and egg removal, together with clutch manipulations, provided the key stimulus and therefore were the most important cues, as increased interaction in addition to clutch manipulation has been shown to increase abandonment in a cuckoo host (Moksnes and Røskaft 1989). Thus, I predicted that visits to the nests to add a cowbird egg before sunrise and remove a host egg after sunrise and to present a cowbird model before sunrise for laying and later the same morning for the egg-removal event would elicit the most rejection of all groups. Abandonment (via burial or desertion) would be recorded most frequently at nests with the most parasitic disturbance (i.e., with all four factors present) and rejection at these nests should be similar to that of naturally parasitize nests.

2. Aggressiveness – I hypothesized that individual variation plays a role in predicting which birds accept or reject parasitism. Individuals vary in the rate of *seet* calling and in the number of strikes on a cowbird model (Campobello 2008). Yellow

Warblers utter *seet* calls preferentially toward cowbirds during their egg-laying period, but *chip* call toward nest predators such as mammals, including humans, and other birds, during the entire nesting period (Gill and Sealy 2004). Indeed, female warblers *seet* called more in the laying stage relative to the nestling stage and gave more *seet* calls in response to *seet* playbacks relative to *chip* playbacks (Gill and Sealy 2004). *Seet* calls may startle cowbirds and deter them from nest searching (Gill and Sealy 2003). This alarm call may also alert other warblers of the parasite's presence (Gill and Sealy 2003). Ninety-seven percent of adults *seet* called during presentation of a laying model after sunrise in a previous study (Sealy *et al.* 1995).

I predicted that in an experimental group involving presentation of a female cowbird model, some warblers would have a higher tendency to attack (i.e., strike the model; more aggressive behavioural type), whereas others would be less aggressive and be less likely to attack (i.e., no striking; less aggressive behavioural type) (Sih *et al.* 2004). I also predicted that females that *seet* called and struck the cowbird models most frequently would be more likely to bury or desert a cowbird egg. Individuals may be more aggressive relative to others across contexts and aggressiveness may be correlated with other behaviours (behavioural syndromes; Sih *et al.* 2004). For example, funnel web spiders (*Agelenopsis aperta*) that are more aggressive attack prey and conspecific territorial intruders sooner than less aggressive individuals (Riechert and Hedrick 1993). Aggressive individuals, however, also partake in more non-adaptive wasteful killings where prey is killed but not consumed (Maupin and Riechert 2001, Sih *et al.* 2004). This concept of behavioural syndromes may be applied to Yellow Warblers. Individuals that are more aggressive, in addition to attacking the cowbird model, may be more likely to

desert their clutch by burying their own eggs, along with the parasite's egg or completely desert their current nesting attempt. Parental behaviour (i.e., incubation, brooding, **not** sacrificing your own eggs in a burial) is often negatively correlated with aggressiveness (Wingfield and Soma 2002). Finally, evidence suggests that host aggression against an adult brood parasite and egg rejection behaviour evolve together and thus accepter species are less aggressive than rejecter species (Røskaft *et al.* 2002).

  3. Timing of parasitism. - It is important to add a cowbird egg before sunrise at some nests and after sunrise at others during the same breeding season to demonstrate experimentally whether there is a shift in rejection behaviour before or after host laying. Based on results from 2008, I hypothesized that adding cowbird eggs to nests before sunrise would elicit rejection. I predicted more rejection would be recorded at nests experimentally parasitized before sunrise and at naturally parasitized nests and these two frequencies would not differ significantly. Nests parasitized after sunrise, however, were predicted to elicit less rejection than the two other groups in accord with Sealy's (1995) report of a low rejection frequency at nests parasitized after sunrise.

  4. Visual versus tactile cues. - I hypothesized that the decision to abandon is based on tactile cues because Yellow Warblers do not recognize their own eggs (Sealy and Lorenzana 1998), usually bury cowbird eggs along with host eggs (Guigueno and Sealy 2009), settle on their clutch before ejecting non-egg-shaped (but not "broken") objects (Guigueno and Sealy 2009), and reject (mostly by burial) non-egg-shaped objects more frequently than cowbird eggs (Guigueno and Sealy 2009). I predicted that warblers whose nests received a cowbird egg in their nest would not visually inspect their clutch upon return longer than warblers at control nests (Sealy and Bazin 1995, Sealy and Lorenzana

1998). Warblers that received a cowbird egg, however, would "shuffle" their bodies (to contact their developing brood patch with the nest contents; see Höhn 1993) more frequently than warblers at control nests and probe the eggs more frequently (Sealy and Lorenzana 1998).

To investigate further the roles of visual and tactile cues, I tested the response of Yellow Warblers to model cowbird-like and non-mimetic blue eggs. Cowbird eggs are similar to warbler eggs in colour, but cowbird eggs are twice the size and mass (see Guigueno and Sealy 2009). Non-mimetic blue and cowbird-like eggs (mimetic in appearance to host eggs) were added to nests of grassland passerines (Klippenstine and Sealy 2008). Blue eggs were grasp- or puncture-ejected more frequently than cowbird-like eggs, which suggests that similarity in appearance between the parasitic and host eggs impeded host discrimination (Klippenstine and Sealy 2008). Ejection of both egg colour types suggests egg recognition on the part of these grassland species, as hosts do not simply eject discordant eggs (Rothstein 1975c, 1978; Underwood and Sealy 2006; Klippenstine and Sealy 2008). Similar results were obtained by Burhans and Freeman (1997) who added cowbird eggs painted white and normal cowbird eggs to nests of Yellow-breasted Chats (*Icteria virens*), which lay eggs similar in appearance to cowbird eggs. White eggs were ejected more frequently (Burhans and Freeman 1997). Marking an egg with a yellow highlighter pen created a highly non-mimetic (and rejected) egg in Great Reed Warblers, a host species that buries and ejects cuckoo eggs (Moskát *et al.* 2009). Kuehn (2009) added two non-mimetic blue eggs to Yellow Warbler nests to increase the magnitude of clutch volume change, which may increase rejection of

experimentally parasitized clutches because volume change may be the cue used by Yellow Warblers to reject foreign eggs (Rothstein 1982b, Kuehn 2009).

Although Yellow Warblers apparently do not eject cowbird eggs, a visual cue may be important in deciding whether to bury or desert. If this is the case, a stronger visual cue may increase the rejection frequency, which would suggest some egg recognition abilities in this species, despite previous work having suggested that they do not recognize their own eggs (Sealy and Lorenzana 1998). If a tactile cue is more important, as I predicted, non-mimetic eggs will be rejected at a similar frequency as cowbird-coloured eggs, as both egg types will presumably "feel" the same to the warblers.

5. *Individual responses over the breeding season.* - Individuals were hypothesized to respond differently to parasitism as the breeding season progresses, burying or deserting after being parasitized at the beginning of the breeding season but accepting parasitism near the end when time has run out (Sealy 1995). Sealy (1995) recorded burial and desertion early in the breeding season, but most females parasitized later stayed with their clutch. To demonstrate experimentally individual change in behaviour as time progresses, I re-tested the response of females that buried experimentally added eggs and naturally laid eggs. During re-parasitism events, less time remained in the breeding season than during the first parasitism event. Therefore, I predicted that females that buried eggs would not all bury again a second time and the rejection frequency at re-parasitized nests should be similar or less than the rejection frequency at all nests parasitized for the first time.

METHODS

Study Site

I conducted my research during May and June 2008 (Experiment 1) and 2009 (Experiment 2) at Delta Marsh, Manitoba, Canada (50°11′N, 98°19′W), on the properties of the Delta Marsh Field Station (University of Manitoba), Portage Country Club, Delta Waterfowl Station, and Bell Estate (MacKenzie 1982, Briskie *et al.* 1992). Delta Marsh is located along the south shore of Lake Manitoba and encompasses marsh habitats and a dune-ridge forest (Mackenzie 1982, Alderson *et al.* 1999). Yellow Warblers at Delta Marsh nest mainly in the forested dune ridge, although a few pairs nest in marsh-edge habitat (Alderson *et al.* 1999). The clutch initiation period extends from late May to early July (Goossen and Sealy 1982). My assistants and I were headquartered at the Delta Marsh Field Station (University of Manitoba).

Study Species

Yellow Warblers and Brown-headed Cowbirds were recorded at Delta Marsh from the time of settlement by Europeans (Thompson 1891) and they are commonly seen in this area every year. This long period of interaction has resulted in strong selection pressure for the evolution of *seet* calls and nest-sitting behaviour (i.e., birds return quickly and sit tightly on their nest) in the Yellow Warbler, which are produced in the presence of cowbirds (Hobson and Sealy 1989, Gill and Sealy 2004). Each year through the mid-1990s, the frequency of parasitism on Yellow Warblers at Delta Marsh averaged around 21%, but it varied from year to year, ranging from 13.6% to 3 1.3% (Sealy 1995). The frequency of parasitism at Delta Marsh is positively correlated with overall host abundance (Woolfenden *et al.* 2004). The availability of cowbird hosts (i.e., host

breeding synchrony) may influence the level of parasitism experienced by other host species (Woolfenden *et al.* 2004). Yellow Warblers are parasitized much more frequently than more suitable hosts such as the Least Flycatcher (*Empidonax minimus*, Briskie and Sealy 1987) because they nest lower and are less aggressive toward female cowbirds at their nests (Briskie *et al.* 1990). Unlike Yellow Warblers, Least Flycatchers accept cowbird eggs, which makes them a better host for raising a brood parasite (Briskie *et al.* 1990).

Cowbirds lay around sunrise, before warblers have laid their own eggs (Scott 1991, Neudorf and Sealy 1994); however, nests have been parasitized experimentally in previous studies after egg laying. Therefore, if a nest is experimentally parasitized when it contains two host eggs, the warbler will lay again approximately one day later, whereas when it is naturally parasitized, the warbler will likely lay another egg later the same morning. Nevertheless, burial usually occurs during the pre-laying and early-laying stages (Sealy 1995). Parasitizing nests early ensures the parasite will hatch with or before host eggs so that it can compete favourably or even outcompete host nestlings for food (McMaster and Sealy 1998, 1999). Yellow Warblers are also more likely to accept cowbird eggs later in the breeding season when less time remains for building a new nest and laying another clutch after burial (Clark and Robertson 1981, Burgham and Picman 1989, Sealy 1995).

Experimental groups

I conducted Experiment 1 in May and June 2008 to test the responses of Yellow Warblers to different cues that may independently or in concert elicit burial or nest desertion (hypothesis 1). Additionally, Experiment 1 revealed whether aggressive

individuals were more likely to reject (hypothesis 2). This experiment included five experimental groups that each contained a before-sunrise component (four treatments and one control, Table 1: all times Central Daylight Time).

Experiment 2 (Table 2) was conducted in May and June 2009 and tested the importance of before versus after sunrise timing of parasitism on rejection frequency (hypothesis 3), importance of visual versus tactile cues used by Yellow Warblers to reject parasitism (hypothesis 4), and consistency of response by individuals over the breeding season (hypothesis 5). Experiment 2 stemmed from results generated in Experiment 1 and comprised three experimental groups of nests tested before sunrise and three others tested after sunrise (Table 2).

Acceptance and rejection criteria

Model cowbird eggs were recorded as accepted if warbler clutches were completed and were tended by the adults for six consecutive days (Sealy 1995). This criterion is a trade-off between excluding rejections related to factors other than parasitism, such as predator interference or inclement weather, and including rejections delayed due to experimental parasitism; less than 1% of rejections responses occur after five days (Rothstein 1982a). Each nest was checked every24 hours after the addition of the model egg or after the initial disturbance (for the control) on LD2 (Sealy 1995). If a model appeared rejected, the nest was inspected for a few more days to confirm the type of response because burial may take more than a day to complete and a nest may seem deserted one day, but the adults may be tending the eggs the next day (Guigueno, pers. obs.). I removed the model egg from the nest when the test was over unless it had been

Table 1. Experimental groups in 2008: all tests were conducted on the day the warblers laid their second egg (LD 2).

Group	Before sunrise (04:15 – 05:30 hrs)	After sunrise (07:00 – 12:00 hrs)
1[1]	Host egg touched	Host eggs touched
2a	Cowbird egg model added	Host eggs touched
2b	Cowbird egg model added	Host egg removed
3a	Laying event simulated[2]	Host eggs touched
3b	Laying event simulated	Egg-removing event simulated[3]

[1] This group was initially separated into 1a and 1b, because 'a' groups (1 to 3) were not going to be disturbed after sunrise. It was necessary, however, to visit nests after sunrise to determine whether the warbler had laid its second egg and if not, this could be interpreted as an early desertion response.

[2] A female cowbird model in the laying position was placed on the nest after a cowbird egg model had been added.

[3] A female cowbird model in the egg-removing position (with artificial host egg in bill) was placed on the nest rim and a host egg was removed.

Table 2. Experimental groups in 2009: all tests were conducted on the day the warblers laid their second egg (LD 2).

Group	Before sunrise (04:15 – 05:30 hrs)	After sunrise (07:00 – 10:00 hrs)
1A[1]	No visit	Host eggs touched
1B	Host egg touched	Nest visited (determine if second egg laid)
2A	No visit	Cowbird egg model added
2B	Cowbird egg model added	Nest visited (determine if second egg laid)
3A	No visit	Blue egg model added[2]
3B	Blue egg model added	Nest visited (determine if second egg laid)

[1] Capital letters differentiate these groups from those in Experiment 1.

[2] Blue egg models differed only in colour from cowbird models.

buried, and examined it for peck marks that would reveal the host had attempted to puncture-eject the egg, following Rothstein (1977).

During daily nest checks, I looked into each nest to see whether a model cowbird egg was gone and, if so, gently pressed the bottom of the nest cup to determine whether it had been buried. I recorded the nest as deserted when the putative parents were not observed close to the nest for three consecutive days and the eggs were cold (Sealy 1992, 1995).

Ejections were excluded from results in Experiment 1 because the two "ejections" were associated with disappearance of host eggs, nest damage and/or adult disappearance. I therefore concluded that these "ejections" were likely due to predation events. Ejections were included in Experiment 2 because, unlike in Experiment 1, models could be punctured (see below), some ejected eggs were found on the ground near the nest and one had a puncture mark, and no nest damage was recorded (see Results).

If burial was recorded at an experimental nest in Experiment 2, the nest was parasitized again the morning the female Yellow Warbler was about to lay her second egg (LD2). For burials at naturally parasitized nests, re-parasitisms occurred at the same stage as when the nest was parasitized the first time. Burials that occurred at nests that were parasitized before nest construction was complete were not re-parasitized because these were likely not true burials (Sealy 1995); warblers were likely finishing lining their nests and the parasitic eggs were covered during the process (Sealy 1995).

Time of manipulations relative to the nesting and breeding cycle

All nests were manipulated on the warbler's LD2, as responses to parasitism change during the nesting cycle and cowbirds frequently parasitize nests on this day or on

the day before (Clark and Robertson 1981, Sealy 1995). Indeed, during laying, cowbirds parasitized most nests on LD2 (Sealy 1995). As cowbirds parasitize most nests prior to laying (Sealy 1995), it would have been best to have parasitized nests at this stage. However, this would be riskier than parasitizing them during laying because Yellow Warblers are more likely to abandon their clutches in response to inclement weather and the time between nest completion and laying the first egg varies between nests (M. F. Guigueno, pers. obs.). By parasitizing nests on LD2, I ensured manipulations were conducted at the same time during the nesting cycle for each nest.

Yellow Warblers are less likely to abandon parasitized clutches later in the breeding season, because acceptance prevents nesting delays associated with nest desertion or burial and there may not be enough time to start again (Clark and Robertson 1981, Sealy 1995). To minimize this effect on desertion frequencies, I conducted the tests (excluding re-parasitism events), up to June 20; that is, during the first half of the breeding season.

Model egg addition

Brown-headed Cowbirds at Delta Marsh lay on average 31.2 ± 2.0 (SE) minutes before sunrise and Yellow Warblers, 13.3 ± 2.8 (SE) minutes after sunrise (McMaster *et al.* 2004, Sealy and McMaster 2004). It was important that I parasitize nests during normal cowbird laying times, but to avoid disturbing warblers when they would normally be laying. Sealy *et al.* (2000) and Sealy and McMaster (2004) together described five acts of parasitism by cowbirds on Yellow Warblers and one apparent attempt. The successful cowbirds arrived at the nests from 33 to 19 minutes before sunrise (average ~ 25 minutes; Sealy *et al.* 2000, Sealy and McMaster 2004), whereas the unsuccessful female, which

flew away from the nest after encountering a roosting female warbler, arrived 43 minutes before sunrise (Sealy *et al.* 2000).

Sunrise times between 29 May (approximate clutch initiation date; based on 2007 data) and 20 June, 2008 and 2009 ranged from 05:23 and 05:30 CDT (Astronomical Applications Department, U. S. Naval Observatory, Washington, DC). Therefore, nests that needed to be parasitized before sunrise had a model egg added between 04:15 and 05:30 hrs CDT (68 to 75 minutes before sunrise to 0 to 7 minutes after sunrise). This interval likely encompasses all times at which cowbirds parasitize Yellow Warbler nests at Delta Marsh, as parasitism times vary little (McMaster *et al.* 2004). I added model eggs to nests that needed to be parasitized after sunrise (Experiment 2 only) between 07:00 and 10:00 CDT (Sealy 1995).

For tests with a before-sunrise component, I inspected nests the afternoon before parasitism to confirm the precise location of each nest, which had been marked with numbered flashing tape. I set up reflectors in vegetation by the trail and along the road to facilitate finding the nest the next morning before sunrise. My assistants and I wore dark green jackets and caps to blend in with the natural environment. As Yellow Warbler nests were located in a long riparian forest habitat along Lake Manitoba and tests had to be conducted within a short period of time before sunrise, numerous assistants were required. Treatments were randomly assigned to assistants by rolling a dice the night before the tests were to begin. Nests naturally parasitized were monitored to determine the rejection frequency for each year and this was compared to the rejection frequency at experimental nests.

Model eggs

We decided to use blue eggs as non-mimetic eggs (Klippenstine and Sealy 2008) because using white eggs may simulate the appearance of fecal sacs (Burhans and Freeman 1997). All model eggs were painted with non-toxic, water-resistant acrylic paints (Folk Art Opaque Acrylic Paints by Plaid[®]; Plaid Enterprises Inc., Norcross, GA, USA; product numbers and colours below) to resemble cowbird eggs or non-mimetic blue eggs, similar to those used by Klippenstine and Sealy (2008). The background of cowbird eggs was off-white (Wicker White 1643 mixed with Licorice 1506) with brownish markings (Burnt Umber 1618 mixed with Wicker White) that varied in diameter (Lowther 1993). The colours were mixed to resemble closely the colouration of real cowbird eggs (at the same time resembling Yellow Warbler eggs, Fig. 1). Realistic markings were created by spattering spots on the objects with a toothbrush dipped in paint. Non-mimetic eggs were painted with Cobalt Blue 1631 mixed with Wicker White 1643 (Fig. 1).

Egg models used in Experiment 2 were slightly different. Egg models in Experiment 1 had a small styrofoam centre so as to match the weight of real cowbird eggs, however, these eggs could not be punctured. Although Yellow Warblers are not known to grasp- or puncture-eject cowbird eggs (Sealy 1995), and were not predicted to do so, a non-mimetic egg used in Experiment 2 differed enough from host eggs to potentially allow the warblers to discriminate between their eggs and the foreign egg and to attempt puncture-ejection of the foreign egg. Therefore, it was important to facilitate egg discrimination by the warblers. Models used in Experiment 2 were made by shaping floral foam into an egg and placing it in a cowbird mould with a thin layer of plaster of

A

B

Figure 1. Parasitized clutches in Experiments 1 and 2. A was used in groups 2a, 2b, 3a, and 3b (Experiment 1) and 2A and 2B (Experiment 2), whereas B was used in groups 3A and 3B (Experiment 2). Each nest contains one model egg (A – normal, B - blue) and three real Yellow Warbler eggs.

Paris (similar to those employed by Lee *et al.* 2005). The dimensions and weights of the models were similar to real cowbird eggs (Table 3). All models were sanded three times before they were painted and each time, finer sandpaper was used to ensure their surfaces were smooth. Each model was heated in the hand for 10 seconds before being placed in a nest and each nest was used only for one test except in the case of re-parasitism events, which were parasitized twice.

Behavioural responses

Experiment 1. - Behavioural responses of Yellow Warblers to laying and egg-removing models (Fig. 2) were recorded using camcorders to determine whether individuals with the strongest response (i.e., more strikes and *seet* calls) were more likely to reject parasitized nests. Laying and egg-removing cowbird models were placed on nests for one minute of interaction or up to 5 minutes if the parents were not seen within 5 m of the nest. Laying lasts about one minute (63 ± 12.3 sec [Neudorf and Sealy 1994], 41.0 ± 4.58 sec [Sealy *et al.* 1995], 21.8 ± 3.9 sec [Sealy *et al.* 2000], 48 ± 6 sec [McMaster *et al.* 2004]) and cowbirds take less than one minute to remove a host egg (Sealy, pers. comm.). Behaviour was recorded at all nests, including the controls, to be consistent across experimental groups. I recorded the warblers' behaviour for one minute from natural cover (Höhn 1993) about 7.5 m from the nest. I waited up to 5 minutes for the adults to appear if they were not in the vicinity of the nest. I video recorded the nest and the area within 5 m of it for one minute if no adults appeared after five minutes. Hosts may silently watch the cowbird model (Cedar Waxwings *Bombycilla cedrorum,* Neudorf and Sealy 1992; Yellow Warblers, Hobson and Sealy 1989, Gill *et al.* 1997).

Table 3. Mean (± SE) (top row) and range (bottom row) of measurements and mass of model eggs added to Yellow Warbler nests in 2008 and 2009, and of a sample of cowbird and warbler eggs.

Egg	*n*	Length (mm)	Width (mm)	Mass (g)
Unpuncturable, cowbird	102	22.10 ± 0.08	17.10 ± 0.08	3.20 ± 0.04
		(19.8 - 23.3)	(15.6 - 18.3)	(2.4 - 3.7)
Puncturable, cowbird	110	22.80 ± 0.04	17.88 ± 0.03	3.00 ± 0.02
		(21.1 - 23.3)	(17.1 - 18.3)	(2.5 - 3.6)
Puncturable, blue	110	22.85 ± 0.04	17.93 ± 0.02	3.01 ± 0.03
		(21.5 - 23.3)	(17.4 - 18.3)	(2.3 - 3.7)
Real cowbird[1]	77	21.07 ± 0.12	16.36 ± 0.09	3.14 ± 0.04
		(18.1 - 23.3)	(14.3 - 18.3)	(2.1 - 3.9)
Real warbler[1]	85	16.80 ± 0.08	12.60 ± 0.04	1.43 ± 0.01
		(14.7 - 18.4)	(11.6 - 13.4)	(1.2 - 1.7)

[1] Data from Sealy (1992).

A

B

Figure 2. Models of female Brown-headed Cowbird (A) in the laying position on a

Yellow Warbler nest and (B) during a host egg-removal event.

Other Yellow Warblers were "out of area" when a female cowbird model was placed at varying distances from their nest (Gill *et al.* 1997: 61) and some warblers left the area or were not seen after Fox Sparrow (*Passerella iliaca*) and cowbird models were presented during different nesting stages (Hobson and Sealy 1989). Therefore, warblers do not always attack cowbird models placed near their nest.

I recorded from the videos the number of *seet* calls and *chip* calls given during one minute. As *seet* calls denote cowbirds, but *chip* calls denote any nest predator (Gill and Sealy 2004), by recording calls I determined whether Yellow Warblers perceived the models as cowbirds before as well as after sunrise. I also recorded the number of times each female Yellow Warbler struck the cowbird model with its bill or body. Before sunrise, built-in infrared light in the camcorders, supplemented with an external infrared light, produced a clear image. Females of some parasitic birds have duller plumage than non-parasitic birds (Payne 1967), suggesting they are less conspicuous. A higher *seet* call rate in the presence of dull-plumaged birds compared with control nests, during normal parasitism times, would suggest that the warblers can recognize their parasites.

Freeze-dried models of female cowbirds were used for treatments 3a and 3b. Two models of females in laying position and two egg-removing models were made from cowbird carcasses, which were correctly positioned, frozen, and freeze dried for about four days. Models in egg-removing posture stood with their bills opened to which artificial Yellow Warbler eggs were attached (Fig. 3A). Their feet were attached to a dowel connected to a radio-controlled servo (Fig. 3B). The boxed receiver (Fig. 3C) and external battery (Fig. 3D) remained on the ground or were attached to a nearby tree or
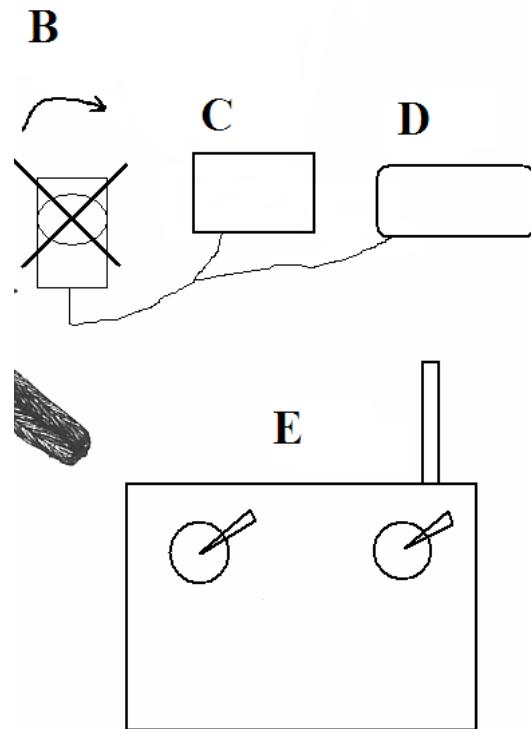
Figure 3. (A) Female Brown-headed Cowbird model with artificial host egg in bill; (B) servo with one extended arm; (C) receiver; (D) external battery, and (E) transmitter with internal battery. Figure of female cowbird taken from Johnsgard (1997: p. 347).

branch; the servo was attached to a nearby branch. Radio-controlled, the egg-removing

model moved upward from an original position of having its head in the warbler's nest.

Experiment 2. - A subset of nests from each group was video recorded to

compare the adult warblers' behaviour before and after the manipulation. Behaviour was

measured approximately 30-45 minutes before a nest visit (control groups) or clutch

manipulation (experimental groups) until approximately 30-45 minutes after the

manipulation. The tripod and camera case were placed about 7.5 m from the nest the

night before to ensure the warblers habituated to the equipment. Attached to the tripods

were clear plastic containers in which camcorders were placed when the recording began.

These containers protected the camcorders from light rain and were partly covered with

burlap, along with the tripod legs, to camouflage the equipment (Fig. 4). Camcorders

were equipped with 12-hour batteries charged daily. Five different behavioural

measurements were analyzed (see Sealy and Lorenzana 1998 for similar comparisons)

including the number of: (1) seconds warblers sat on the nest rim directing their gaze

toward their eggs, (2) visits to the nest before settling on the clutch, (3) times probing the

eggs before settling on the clutch, (4) times the females "shuffled" their bodies once

settled on the clutch divided by the time settled on the clutch (Höhn 1993) and, (5)

proportion of time probing the eggs with the bill once settled on clutch. Camcorders

recorded behaviour at least one-half hour before the manipulation and about 4-6 hours

after the manipulation. The longer recording time after parasitism was intended to record

warblers that brought nest material to bury their newly parasitized clutch, as this may

occur within hours of being parasitized. Burial has been recorded on the day after

parasitism in previous clutch manipulation studies (Guigueno and Sealy 2009, in press).

<div align="center">A</div>

<div align="center">B</div>
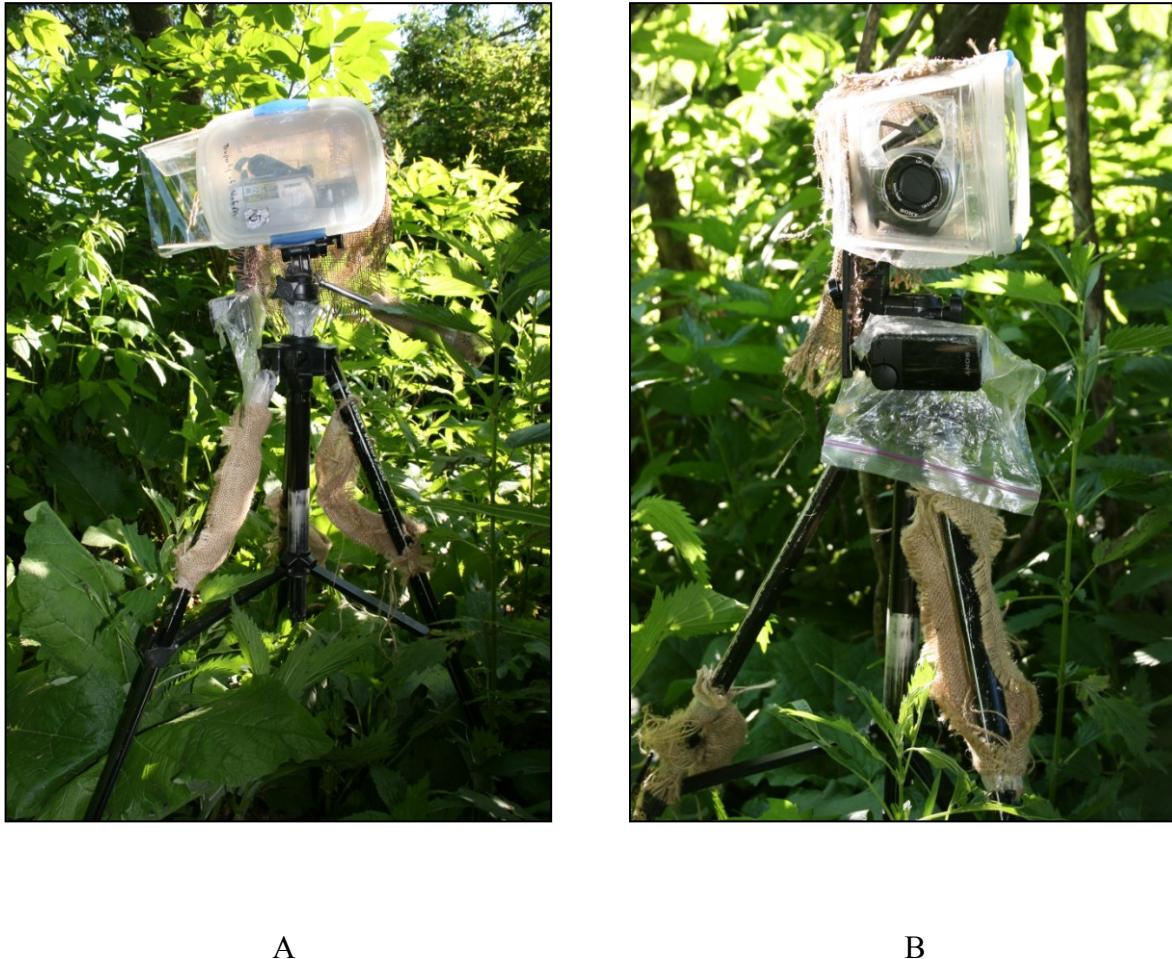
Figure 4. Side (A) and front (B) views of the camcorder setup in 2009. The infrared light is attached below the camcorder case and protected by a plastic bag.

Statistical analyses

Experiment 1. - I used a Fisher's exact test as more than 20% of the cells had

expected values less than five to compare the highest rejection frequency recorded at an

experimental group with the rejection frequency at naturally parasitized nests (Zar 1999).

To determine which factors influenced rejection in warblers, I used the "PROC

LOGISTIC" procedure in SAS (SAS Institute Inc., Cary, North Carolina, 1990) and

performed logistic regression on four predictor categorical variables (add egg, remove

egg, laying cowbird model, egg-removing model) (Kosciuch *et al.* 2006). Models

predicted the probability of rejection given the presence or absence of each categorical

variable (Table 4). I used Akaike's Information Criterion corrected for small sample sizes

(AIC_C) to rank various general linear models using logit link functions (Kosciuch *et al.*

2006):

AIC_C = Dev + 2K + (2K[K+1]) / (n-K-1)

where: Dev = model deviance (-2 ln *L*)

K= # of parameters in the model

n = sample size

(2K[K+1]) / (n-K-1) = correction for small sample sizes

I used a backward stepwise logistic regression based on AIC values to select a

best-supported model. The most parsimonious model had the lowest AIC_C value (ΔAIC_C

= 0), however, additional models with $\Delta AIC_C \leq 2$ were considered equally as

parsimonious (Anderson *et al.* 2000, Kosiuch *et al.* 2006). Also, the strength of support

for each model was determined by calculating Akaike weights (ω_i):

$\omega_i = e^{(-\Delta i/2)} / (\Sigma^R_{r=1} e^{(-\Delta r/2)})$

Table 4. Each group in Experiment 1 represented by the absence (0) or presence (1) of categorical variables. See Table 1 for verbal description of groups.

Group	Add egg	Remove egg	Laying cowbird[1]	Egg-removing cowbird[1]
1	0	0	0	0
2a	1	0	0	0
2b	1	1	0	0
3a	1	0	1	0
3b	1	1	1	1

[1] Models.

where:

Δ_i = ΔAIC_C of the model in question divided by the sum of all other candidate models (Δ_r).

The direction and strength of each effect were determined from the odds ratio (e^{β}). An odds ratio < 1 indicates the factor reduced the odds of an effect and an odds ratio > 1 indicates it increased the odds of an effect, whereas an odds ratio = 1 indicates the factor has no effect (Stokes *et al.* 2000, Kosiuch *et al.* 2006). The use of AIC values (as opposed to *p*-values) is based on the information theoretic approach developed by Anderson *et al.* (2000, 2001). In this approach, scientific statements about nature and the weight of models are based on information available rather than on arbitrary *p*-values used in the frequentist approach (hypothesis testing), developed by K. R. Popper (Anderson *et al.* 2001). AIC values have been used in recent behavioural studies, including one on nest desertion (Kosiuch *et al.* 2006) and, hence I incorporated this approach.

Seet calls and strikes were recorded in Group 3b and to determine whether there were differences in the number of strikes, *seet* calls, and *chip* calls between individuals that rejected or accepted parasitism, exact logistic regression was used because the sample sizes in each group (accepted versus rejected) were small and thus the data did not fit a regular logistic model (Hirji *et al.* 1987, Stokes *et al.* 2000). Hirji *et al.* (1987) programmed this technique, which was originally devised by Cox (1970), for SAS within the "PROC LOGISTIC" procedure. Analysis of small, sparse, and skewed data is frequently unreliable if general linear models, mixed-effect models, or other asymptotic methods of analysis are used (Derr 2000, Stokes *et al.* 2000). AIC values, however, cannot be computed in exact logistic regression, therefore, each behavioural measurement

(strike, *seet* call, and *chip* call) was analyzed in a separate model and the exact odds ratios were interpreted to determine the direction and strength on rejection of each behavioural characteristic.

Experiment 2- Exact logistic regression was used to analyze (1) the importance of introducing eggs to nests, (2) introducing eggs to nests before sunrise, and (3) introducing non-mimetic blue eggs on the probability of rejection (Table 5). The data did not fit a regular logistic model because the maximum likelihood estimate, which is used to fit a statistical model to the data, may not have existed due to a quasi-complete separation of data points (Hirji *et al.* 1987, Stokes *et al.* 2000). Exact parameter estimates (ß) were reported for each categorical variable, along with 95% confidence intervals and *p*-values because AIC values cannot be computed in exact logistic regression. Predictions three and four would be satisfied if "add egg" and "before sunrise" are the only significant factors. "Add non-mimetic egg" would not be a significant factor because I predicted that cowbird-like and non-mimetic eggs would be rejected at similar frequencies (Hypothesis four). To compare the rejection frequencies at the groups represented by the significant factors to the rejection frequency at naturally parasitized nests, a 5 x 2 chi-square test in SAS was used. Finally, the method of rejection (burials and desertion versus ejection) between groups receiving cowbird eggs and non-mimetic blue eggs was analyzed using a 2 x 2 Fisher's exact test because one of the cells contained a frequency of zero (Zar 1999).

The differences in video-recorded behaviours before versus after manipulations were compared between control groups and the experimental groups using Wilcoxon rank

Table 5. Each group in Experiment 2 represented by the absence (0) or presence (1) of categorical variables. See Table 2 for a verbal description of groups.

Group	Before sunrise	Add egg	Add non-mimetic egg
1A[1]	0	0	0
1B	1	0	0
2A	0	1	0
2B	1	1	0
3A	0	1	1
3B	1	1	1

[1] Capital letters differentiate these groups from those in Experiment 1.

sum tests because the data were not normally distributed, even after a log transformation. *P*-values were reported; those significant after a sequential Bonferroni correction (Rice 1989) were identified.

Nests at which burial or ejection was recorded were parasitized again and the second response was recorded to determine whether the same individual's behaviour changes as the breeding season progresses. I compared the frequency of rejection at nests after the first parasitism and after the second using 2 x 2 Fisher's exact tests because more than 20% of the cells had an expected value of less than five (Zar 1999). Throughout the text, mean ± standard errors are reported. All *p*-values ≤ 0.05 were considered significant.

RESULTS

Experiment 1

A total of 158 experimental nests (including two "ejections") survived for six days (Table 6) and were distributed along approximately 8 km of habitat (Appendix). Warblers likely did not attempt to puncture-eject accepted models because none showed peck marks. The "ejections" would have been included in the analyses if the model eggs had disappeared, but the host eggs remained tended by the warblers (Rothstein 1975a). However, the model eggs were probably not ejected because: (1) the model disappeared at one nest between the fifth and sixth daily checks and the adults were not seen on days five and six and, (2) at the other nest, the model went missing on the fifth day but the nest was damaged. A predator likely "ejected" these model eggs. Therefore, I did not include "ejections" from Experiment 1 in my statistical analyses.

Table 6. Responses of Yellow Warblers to Experiment 1. See Table 5 for groups.

Group	Rejection[1]		Acceptance	Total nests	Rejection frequency (%)
	Burial	Desertion			
1: control	1	1	54	56	3.6
2a: add egg	6	1	15	22	31.8[2]
2b: add + remove eggs	2	1	20	23	13
3a: laying model	1	0	22	23	4.3
3b: laying + egg-removing models	3	3	26	32	18.8

[1] Two "ejections" were recorded. Group 2a: Artificial cowbird egg disappeared on the 6th daily check, but the adults had not been seen on days 5 and 6. Group 3a: Artificial cowbird egg disappeared on the 5th daily check, but the nest was damaged on day 5 or 6.

[2] Similar to that observed at naturally parasitized nests for 2008 (27%, $n = 11$).

The most rejections occurred in group 2a, with abandonment recorded at about the same frequency as at naturally parasitized nests (Table 6); these frequencies did not differ significantly (Fisher's exact test, two-tailed, $p = >0.999$). The top logistic regression model (Δ AIC$_C$) contained all four factors (Table 7). Models with "Add egg" received 98% of the support among models, "Laying cowbird model" received 74%, "Egg-removing model" received 56%, and "Remove egg" received 29% of the support (Table 7). The only supported single factor model contained "Add egg." Four models were considered equally as parsimonious (Δ AIC$_C$ ≤ 2) and their parameter estimates are summarized in Table 8. The logistic equation from the top model is:

Logit (P) = -3.30 + 2.53(Add egg) – 1.14(Remove egg) – 2.33(Laying model) + 2.76 (Egg-removing model)

The positive ß estimates for "add egg" and "egg-removing model" indicate the probability for rejection increased if these factors were present. Warblers whose nests received an egg before sunrise were 12.60 x (95% CI = 2.37, 67.10) more likely to reject parasitism than warblers that did not receive an egg before sunrise. Warblers at nests at which egg-removing cowbird models were presented were 15.80 x (95% CI = 1.10, 226.06) more likely to reject parasitism. The negative ß estimates for "remove egg" and "laying model" indicate the probability for rejection decreased if these factors were present. Warblers at nests at which the laying model was presented were 0.10 x (95% CI = 0.01, 0.88) as likely to reject parasitism and warblers at nests from which a host egg was removed were 0.32 x as likely to reject parasitism but the 95% CI surrounding this estimate included one (0.07, 1.45).

Table 7. Logistic regression model results for the probability of rejection for Yellow

Warblers in Experiment 1.

Logistic regression model	AIC_C[1]	ΔAIC_C[2]	w_i[3]
1. Add egg + Remove egg + Laying cowbird model + Egg-removing cowbird model	112.10	0.00	0.29
2. Add egg + Laying cowbird model + Egg-removing cowbird model	112.31	0.20	0.26
3. Add egg	112.51	0.41	0.24
4. Add egg + Laying cowbird model	113.02	0.91	0.19
5. Intercept only (null model)	117.62	5.52	0.02

[1]AIC corrected for small sample sizes.

[2]Difference between subordinate and top models.

[3]Akaike weights.

Table 8. Parameter estimates (ß ± SE) for all parsimonious models (Δ AIC$_C$ $\leq$ 2) in Experiment 1.

Model factors	Model 1	Model 2	Model 3	Model 4
Add egg	2.53 ± 0.85	2.04 ± 0.80	1.71 ± 0.77	2.04 ± 0.80
Remove egg	-1.14 ± 0.77	n/a	n/a	n/a
Laying model	-2.33 ± 1.12	-1.84 ±1.08	n/a	-0.67 ± 0.54
Egg-removing model	2.76 ± 1.36	1.62 ± 1.12	n/a	n/a

Laying cowbird models presented before sunrise did not elicit attacks or *seet* calls. Most warblers (83.93%, *n* = 55) flushed from their nests and none was seen < 5 m from its nest (i.e., they moved out of sight) after laying models were positioned. However, the laying model affected rejection frequency, described above. Egg-removing models presented after sunrise elicited attacks, *seet* calls, and *chip* calls from some individuals. Individuals that buried or deserted *chipped* less than those that accepted (ß = -0.5039, *p* = 0.0467). For every *chip* call, individuals were 0.604 x (95% CI = 0.117, 0.998) as likely to reject. However, the number of strikes (ß = 1.6609, *p* = 0.0605) and *seet* calls (ß = 0.0905, *p* = 0.1323) had little effect on the probability of rejection. For every strike, individuals were 5.264 x (95% CI = 0.955, ∞) more likely to reject and for every *seet* call, individuals were 1.095 x (95% CI = 0.977, 1.251) more likely to reject, but the confidence intervals include one.

Results differed, however, when desertions are removed from rejections and acceptances are compared to burials only. Individuals that buried struck the model more than individuals that accepted (ß = 2.566, *p* = 0.0148). For every strike, individuals were 13.010 x (95% CI = 1.338, ∞) more likely to bury. Individuals that buried *seet* called more than individuals that accepted (ß = 0.1389, *p* = 0.0307). For every *seet* call, individuals were 1.149 x (95% CI = 1.011, 1.359) more likely to bury, however, the number of *chip* calls (ß = -0.255, *p* = 0.4431) had no effect on the probability of burial. For every *chip* call, individuals were 0.775 x (95% CI 0.185, 1.056) as likely to bury, but the confidence interval includes one.

Experiment 2

A total of 216 experimental nests survived for six days (Table 9). No eggs from nests at which acceptance was recorded were pecked. The highest rejection frequency was elicited in group 3B, where non-mimetic eggs were placed in nests before sunrise, but it did not differ significantly from the frequencies of the other groups (2A, 2B, and 3A) and at naturally parasitized nests (5 x 2 chi-square test, $\chi^2 = 2.9281$, df $= 4$, $p = 0.5699$; Table 9). In the exact logistic model, only "Add egg" was significant (Table 10). However, only ejections were recorded in groups 3A and 3B (Table 9). The differences in rejection methods (burials/desertions versus ejections) between groups receiving a cowbird egg (2A and 2B) and groups receiving a blue egg (3A and 3B) approached significance (2 x 2 Fisher's exact test, two tailed, $p = 0.0645$).

The difference in behaviour of warblers before versus after a manipulation differed significantly between controls (1A and 1B) and treatments (2A, 2B, 3A, and 3B) (Table 11). Individuals returning to their nests after an egg had been added peered longer at their eggs, shuffled their bodies more frequently once settled on their clutch, and spent more time probing eggs with their bills compared to controls (Table 11).

Individuals that buried an experimental or naturally laid egg were re-parasitized. Nests parasitized for the first time received an egg on average 7.31 days $\pm$ 0.20 (SE) after clutch initiation and re-parasitism occurred 12.71 days $\pm$ 0.70 (SE) after clutch initiation. Of 19 nests with re-parasitism results, one burial and 18 acceptances (5.2%) were recorded, a frequency smaller but not significantly different from the 21.4 % burial recorded at all nests parasitized for the first time (Fisher's exact test, two-tailed, $p = 0.1260$).

Table 9. Responses of Yellow Warblers to Experiment 2.

Groups	Rejection			Acceptance	Total nests	Rejection (%)
	Burial	Desertion	Ejection			
1A[1]: control	0	0	0	37	37	0.0
1B: control	0	0	0	35	35	0.0
2A: cowbird egg	7	1	0	30	38	21.1[2]
2B: cowbird egg	6	1	0	25	32	21.9[2]
3A: blue egg	7	0	1	24	32	25.0[2]
3B: blue egg	9	1	5	27	42	35.7[2]

[1]"A" represents an after-sunrise manipulation, whereas "B" represents a before-sunrise manipulation.

[2]Similar to that observed at nests naturally parasitized during laying (33.3%, $n = 6$).

Table 10. Exact parameter estimates for a logistic model containing all three categorical

variables from Experiment 2.

Model factor	Estimate (ß)	95% confidence limits		*P*-value
		Lower	Upper	
Before sunrise	0.3036	-0.5121	1.1339	0.5448
Add egg	3.2984[1]	1.4874	∞	< 0.0001
Add non-mimetic blue egg	0.4647	-0.3516	1.3029	0.3043

[1] Median unbiased estimate.

Table 11. *P*-values of Wilcoxon rank sum tests (two-tailed) analyzing differences in behaviour before versus after manipulations between control (1A, 1B) and treatment (2A, 2B, 3A, 3B) groups in Experiment 2.

Variable	Group	*n*	Mean score	S	*P*-value
Peering time (sec)	Control	14	10.21	143.00	<0.0001[1]
	Treatment	26	26.04		
Number of visits to nest before settling	Control	14	18.36	257.00	0.0579
	Treatment	31	25.10		
Number of probes at eggs before settling	Control	14	15.61	218.50	0.0464
	Treatment	26	23.13		
Number of times shuffling / time on clutch	Control	14	12.32	172.50	0.0001[1]
	Treatment	29	26.67		
Proportion of time probing eggs when on clutch	Control	14	14.71	206.00	0.0073[1]
	Treatment	29	25.52		

[1] *P*-values that remained significant after a sequential Bonferroni correction (Rice 1989).

Nests were also re-parasitized after six ejections, with one burial and one acceptance recorded. All other re-parasitized nests were depredated. Of seven ejections (including one at a re-parasitized nest), two host eggs at two nests and one host egg went missing at two other nests. No host eggs went missing at the other three nests. Ejection cost, on average, was 0.86 host egg. Time and energy were not lost, as is the case with burials and desertions. After ejections, adults continued to lay or incubated their clutch and no nest damage was recorded. At three nests, ejected non-mimetic eggs were recovered on the ground 0.3 m, 0.7 m, and 1.0 m from their respective nests. One recovered model had a single puncture mark, approximately 2 mm in diameter.

DISCUSSION

Influence of cowbird-host interactions and clutch manipulations on rejection

In Experiment 1, adding a cowbird egg to nests and interaction with the egg-removing cowbird model increased the probability of rejection, whereas the presence of a laying cowbird model decreased the probability of rejection. Removing a host egg was the least important factor in the logistic model. The most perplexing result is that the laying cowbird model elicited the opposite response to what was expected.

Adding a model egg before sunrise (group 2a) elicited the most rejection among treatment groups, whereas adding a model egg in addition to placing a laying cowbird model on the nest (group 3a) elicited the lowest rejection frequency (Table 6). This is suggestive of warblers rejecting few of the parasitized clutches in group 3a because of possible retaliatory behaviour by the Brown-headed Cowbird (Mafia Hypothesis; Soler *et al.* 1995, Hoover and Robinson 2007). According to this hypothesis, parasites such as the

Brown-headed Cowbird and Great Spotted Cuckoo (*Clamator glandarius*) revisit nests they have parasitized and upon detection of rejection by the host, the parasite destroys the host's nest. Therefore, compliant hosts (i.e., hosts that make the decision to accept parasitic eggs) escape nest destruction by the parasite and individuals that had their nest destroyed will presumably no longer reject parasitized clutches (Soler *et al.* 1995, Hoover and Robinson 2007). However, cowbirds infrequently re-visit nests they have parasitized on our study site (Gill *et al.* 2008), rejection at naturally parasitized nests, where hosts may witness laying event, is more frequent than in group 3b (this study, Sealy 1995), and parasitized nests at which cowbird eggs are buried do not fail at a higher frequency than nests at which acceptance occurred (McLaren and Sealy 2000). Therefore, I reject this possibility.

In group 3b, most females (83.93%, *n* = 55) were flushed from their nests before sunrise when the laying model was placed on their nest and none returned to attack the laying model. In a previous study, more than half the females roosted in their nest overnight and spent on average 28 minutes in their nests during the 30-minute "critical period" when cowbirds were most likely to parasitize nests (Neudorf and Sealy 1994). However, by flying away in low light and not returning, warblers may not have recognized the model as a cowbird. In nature, cowbirds are likely intercepted by as they approach nests. Some adults attacked the laying cowbird in observed natural parasitism events on Yellow Warblers (Sealy 1995, Sealy *et al.* 2000). The warblers in my study may not have seen the laying model, however, it affected rejection, suggesting the warblers did see it (if it were unobserved one would expect no effect), but they may not have recognized it as a brood parasite. We expect the rejection frequencies between

groups 2a and 3a to be similar (Table 1) if the warblers had not seen the laying model, however, the rejection frequency in group 2a was higher than in group 3a, suggesting that the laying model decreased the probability of rejection. Warblers may have perceived the laying cowbird model as nonspecific stressor (i.e., an unknown threat and not a cowbird) and may have "ignored" the potential cue to reject due to a higher stress level, as other animals have been shown to forgo non-essential behaviours in similar situations (Siegel 1980, Whittow 2000, Korte *et al.* 2005). Therefore, a mobile cowbird model such as a robot or a trained cowbird would better simulate natural cowbird laying. It is difficult to know whether interaction with a laying cowbird in a natural context influences the decision to reject. Interaction with a laying cowbird after sunrise elicited few rejections (8.3%) in a previous study (Sealy 1995), which suggests that it may not be as important as interaction with an egg-removing cowbird.

The egg-removing cowbird model, unlike the laying cowbird model, had a positive effect on egg rejection and it elicited more aggression than the laying model (Campobello 2008). It is presumably the egg-removing model, not the removal of a host egg per se, that served as a cue for rejection. This is consistent with Sealy's (1992) results that showed that the probability of warblers accepting the parasitic egg was not affected by the removal of a warbler egg at the time of parasitism. The effect of the egg-removing model supports the hypothesis that increased interaction with the adult parasite in combination with addition of a parasitic egg increases the probability of rejection (Davies and Brooke 1988, Moksnes and Røskaft 1989, Moksnes *et al.* 2000, Yasukawa and Werner 2007). The presence of a cuckoo model promoted host egg discrimination behaviour (Davies and Brooke 1988, Moksnes and Røskaft 1989, and Moksnes *et al.*

2000). It is unlikely that a witnessed egg-removal event alone elicits burial, as Yellow Warblers rarely (6 out of 1885 nests examined; Sealy 1995) bury their own eggs (Sealy 1995). Adding a model egg to a nest is essential in eliciting rejection, because "Add egg" was the most important factor in the logistic rejection model in Experiment 1. Approximately one in three (33%) cowbirds remove a Yellow Warbler egg from nests they have parasitized (Sealy 1992) and rejection occurs at ~ 40% of parasitized nests, depending on the year (see Results; Sealy 1995). The similarity between egg-removal and rejection frequencies and results from Experiment 1 suggest that interaction with an egg-removing cowbird, along with the addition of a cowbird egg to nests before sunrise, are important cues used by warblers to decide whether to abandon parasitized clutches.

Is aggression correlated with egg rejection?

Aggressive individuals, as measured by their interactions with the cowbird model, were more likely to bury the cowbird egg, which supports the prediction that aggression and rejection are correlated. Indeed, aggression against the adult parasite and egg rejection behaviour evolve together and thus accepter species are less aggressive than rejecter species (Røskaft *et al.* 2002). This can be applied to between-individual comparisons within the same species. Yellow Warblers are intermediate egg rejecters. Rejecters may be more experienced and recognize a cowbird as a threat and are more aggressive (Sealy 1995). Sealy (1995) did not find a difference in rejection between yearling versus older female warblers, however, age does not necessarily mean an individual is more experienced because not all nests are parasitized every year (parasitism frequency varied from 14% to 31% between 1974 and 1987; Sealy 1995). A key test

would be to compare aggression and rejection responses between experienced and inexperienced individuals.

Some individuals *chip* called during the presentation of the egg-removing model. This suggests the *chip*-calling individuals did not focus on the immediate threat at their nest (cowbird removing an egg), but possibly focused on us. Despite hiding behind natural cover (Höhn 1993) and wearing clothing that blended with the natural environment, warblers may still have been aware of our presence. We could not use blinds because of time constraints, however, low levels of *chip* calling by Yellow Warblers have previously been recorded in the presence of a cowbird model in which blinds were used in other studies (Hobson and Sealy 1989, Gill and Sealy 2004), thus blinds may not have reduced the amount of *chip* calling. Our results suggest that individuals that were more focused on their nests (i.e., *chip* called less), were more likely to reject parasitism. They may have been more experienced with the threat of parasitism and reacted by *seet* calling and striking the cowbird instead of *chip* calling. Indeed, Yellow Warblers in naïve (less experienced) populations *seet* called less frequently in response to a cowbird model than individuals in sympatric (more experienced) populations (Kuehn 2009).

Are Yellow Warblers more likely to reject if parasitized before sunrise?

When Sealy (1995) parasitized Yellow Warbler nests after sunrise on LD2, the rejection frequency (8.5%) was much lower than at naturally parasitized nests (48.6%). In Experiment 1, adding model eggs to nests before sunrise elicited a rejection frequency similar to naturally parasitized nests (Table 6), however, model eggs were not added to nests after sunrise. I expected similar results with cowbird-like and non-mimetic eggs in

Experiment 2 for eggs added before sunrise only, with eggs added after sunrise predicted to be rejected at a lower frequency, similar to the frequencies recorded by Sealy (1995). My results did not support these predictions (Table 9). Rejection frequencies at nests experimentally parasitized after sunrise were similar to nests experimentally parasitized before sunrise. This begs the question why there was a difference between experimentally and naturally parasitized nests in Sealy's (1995) study.

Methodological differences may explain the discrepancy. For example, Sealy (1995) used actual cowbird eggs while I used models, although it is difficult to see how that would lead to *reduced* rejection frequencies unless the model eggs were being rejected as a manifestation of nest sanitation behaviour (Guigueno and Sealy 2009). Furthermore, Stewart (2003) showed that rejection of real American Robin (*Turdus migratorius*) and model Black-billed Cuckoos eggs, which are similar in size, weight, and colour, was identical in Yellow Warblers. If the differences are real and not just due to methods, there are at least two possibilities.

First, phenotypic plasticity may play a role, especially if environmental cues (weather, changes in vegetation, food supply, etc…) were different in 1988-1991, when Sealy (1995) conducted his study, and this study in 2008-09. Phenotypic plasticity can drive or impede genetic evolution (Price *et al.* 2003). Moderate levels of plasticity are ideal for facilitating genetic evolution, however, plasticity may be either incidental or essential in population differentiation, therefore, individual cases must be examined carefully (Price *et al.* 2003). Moderate levels of plasticity arise when a composite trait, formed by plastic and non-plastic traits, is involved in an adaptive response. However, a

high level of plasticity may impede genetic change because a highly plastic response already places a population near a peak (Price *et al.* 2003).

Mazerolle *et al.* (unpublished data) have shown that Yellow Warblers nesting at Delta Marsh were flexible in their dates of clutch initiation and spring arrival, which were highly variable and correlated with mean May temperatures. This plasticity could carry over into their rejection responses, although Sealy's (1995) data do not support this hypothesis. The rejection frequency in years with late clutch initiation dates (June 9 or later; 60.7%, n = 51 clutches) was similar to the frequency in years with early or normal clutch initiation dates (May 12 – June 1; 44.8%, n = 249 clutches) (chi-square test with continuity correction, χ^2 = 1.80, df = 1, p = 0.18; Sealy 1995).

Conditions on the wintering grounds may affect body condition of warblers returning to breed. Food availability and survival of migratory songbirds increases with wetter conditions on wintering grounds (Sillett *et al.* 2000, Strong and Sherry 2000). Individuals arriving on their breeding grounds may differ in body condition between different breeding seasons. Body condition has been shown to influence nest desertion in Penduline Tits, a species in which both sexes sequentially mate within a breeding season (Bleeker *et al.*2005; see Introduction). The female or male incubates and rears the brood while the other deserts the current nest to breed with another mate. Bleeker *et al.* 2005 reported that individuals in good body condition deserted their nest more often than individuals in poor condition. In hosts of brood parasites, individuals in poor body condition may accept a parasitized clutch or may need more cues to abandon, such as interaction with a cowbird, whereas individuals in good body condition may have enough energy to abandon

their current clutch and re-lay only in response to the presence of a cowbird egg. Indeed, mean body mass of Yellow Warblers varies between years and weeks and yearling warblers are lighter (i.e., in poorer condition) than older individuals (Biermann and Sealy 1985). Measurements of body mass, fat reserves and haematocrit values (Bleeker *et al.* 2005) are required in conjunction with experimentally parasitizing nests to determine whether condition influences the decision to abandon in Yellow Warblers or other hosts that abandon parasitized clutches.

Kuehn (2009) examined phenotypic plasticity and relaxed selection in multiple cowbird hosts: American Robins (*Turdus migratorius*) and Gray Catbirds (*Dumetella carolinensis*), both ejecters, and Yellow Warblers. He recorded the rejection frequency of hosts breeding in sympatric populations (i.e., in the presence of cowbirds), in naïve populations (i.e., where cowbirds are locally uncommon), and in allopatric populations (i.e., in long-term isolation from cowbirds). Robins and catbirds ejected foreign eggs at similar frequencies in sympatric and naïve populations, but at a lower frequency in allopatric populations, suggesting that the lower ejection frequency in the last group is due to evolutionary differences. However, Yellow Warblers abandoned experimentally parasitized nests less frequently in naïve populations than in sympatric populations located nearby, suggesting that abandonment of parasitized clutches is plastic and increases with cowbird exposure. Innate components of abandonment in warblers have persisted for about 6,300 years in isolation from cowbirds because warblers from allopatric populations abandoned parasitized nests at the same frequency as warblers from naïve populations (Kuehn 2009). Similarly, Briskie *et al.* (1992) recorded a lower

abandonment frequency of experimentally parasitized clutches and Gill and Sealy (2004) recorded less *seet* calling in response to cowbird models in allopatric versus sympatric populations of Yellow Warblers.

Phenotypic plasticity by social learning is another mechanism by which hosts may increase nest defence against brood parasitism in a relatively short time (Campobello 2008, Davies and Welbergen 2009). Reed Warblers increased the intensity of nest defence after witnessing conspecifics uttering alarm calls, however, a change in nest defence was not recorded in Yellow Warblers (Campobello 2008). Similarly, Davies and Welbergen (2009) showed that Reed Warblers that witnessed their neighbours mobbing cuckoos increased mobbing of cuckoos but not of a control (parrot), but individuals that did not witness mobbing did not increase mobbing of either the cuckoo or parrot. Therefore, social learning, which may affect how quickly hosts lose or gain defences, influences population dynamics of both hosts and parasites (Campobello 2008, Davies and Welbergen 2009, Takasu *et al.* 1993).

The second possibility that the Yellow Warblers' ability to recognize a parasitized clutch has undergone genetic changes (natural selection) over the 18-21 years between Sealy's (1995) observations and mine. The frequency of rejection at naturally parasitized nests, however, was similar in the two studies, therefore, I am not hypothesizing that there has been a change in the frequency of "rejecter genes" in the population (Rothstein 1975b); rather, I am hypothesizing that warblers that reject are now better able to recognize a parasitized clutch, leading to the increased rejection frequency of experimental eggs only (i.e., not naturally laid eggs). For example, much of the dense forest habitat has disappeared at Delta Marsh, with the number of Yellow Warblers

decreasing (S. G. Sealy, pers. comm.), therefore the only warblers left may be "open area" birds that are genetically more pre-disposed to recognize and respond to a parasitized clutch. Individuals nesting in more open habitat may be more likely to reject an experimentally parasitized clutch because of a historically higher level of cowbird activity in more open areas (Hosoi and Rothstein 2000).Therefore, the individuals tested in my study may have only represented a proportion of individuals tested in Sealy (1995). This is further supported by the differences between my own observations and Sealy and Lorenzana's (1998) observations. As I will argue below, warblers are now able to detect the presence of the parasitic egg in their nest both tactilely and visually, whereas Sealy and Lorenzana (1998) found Yellow Warblers did not recognize their own eggs, and thus were unable to recognize the cowbird egg. Therefore, warblers in Sealy (1995) relied more heavily on interaction with a cowbird to determine they had been parasitized than individuals in the present study. If better egg recognition has evolved, we expect that Yellow Warblers may eventually eject cowbird eggs, possibly by puncture ejection because their bill may be too small for grasp ejection (Hosoi and Rothstein 2000, Rasmussen 2008).

It is difficult to determine whether the discrepancy in the rejection of experimentally added eggs in Sealy's (1995) study and mine is due to phenotypic plasticity (more likely) or genetic changes. Indeed, it is possible that changes in egg recognition described above could also be due to phenotypic plasticity. These two possibilities should be interpreted with caution because further work needs to be conducted to better understand this variation in rejection of experimental eggs. Sealy (1995) conducted clutch manipulations over four years, whereas my work was only

conducted over two years but only one year involved adding an egg model after sunrise. To support a conclusion, (1) clutch manipulations would need to be conducted over many more years, (2) the adult warblers' age and body condition would need to be measured, (3) weather and vegetation changes on the wintering and breeding grounds accessed, and, (4) nests would need to be monitored for cowbird visits (Gill *et al.* 2008) and social learning from conspecifics. If the abandonment frequency of experimental eggs increases over multiple years and this increase is not correlated with the any of the factors above, there would be more support for genetic change (Price *et al.* 2003, Davies and Welbergen 2009, Kuehn 2009).

Do Yellow Warblers reject parasitism on the basis of tactile or visual cues?

Two separate tests were conducted in Experiment 2 to determine whether Yellow Warblers use tactile or visual cues to decide whether to reject a parasitic egg: addition of cowbird-sized non-mimetic eggs (Table 9) and video-recorded behavioural responses of warblers before and after manipulations (Table 11). Sealy and Lorenzana (1998) concluded that Yellow Warblers do not recognize their own eggs because there was no change in behaviour before versus after egg addition and Yellow Warblers did not reject clutches with blue warbler-sized eggs and clutches with different proportions of cowbird and host eggs. By contrast, I obtained two sets of observations that suggest both visual and tactile cues are associated with the decision to reject parasitism in Yellow Warblers. This is the first study to demonstrate that both tactile and visual cues play a role in eliciting rejection in a host of a brood parasite.

First, warblers peered longer at their clutch upon return after a cowbird or blue egg had been added than at control nests (see also Bazin and Sealy 1995). This suggests

they visually recognized a change in their clutch contents and likely recognized the presence of an egg different from their own in their clutch (Table 11). Warblers did not peer longer because of human visits to nests because the change in behaviour before versus after the manipulation was compared between control and treatment groups. Although non-mimetic eggs were rejected at a similar frequency as cowbird eggs (Table 9), only non-mimetic eggs were ejected from nests, which confirms that some warblers visually recognized the parasitic egg. Although not statistically significant, 31.1% of non-mimetic eggs were rejected compared to 21.4% of cowbird eggs, an increase of 45%. Peering time and responses to non-mimetic eggs strongly suggest that visual cues are used by Yellow Warblers to decide to reject a parasitized clutch. Sealy (1995) recorded instances of ejections of real cowbird eggs, but none of the ejected eggs was found near the nest. Rather, Sealy (1995) believed partial depredation was the cause of the "ejections." Thus, my observations of ejected non-mimetic eggs constitute the first support that Yellow Warblers also eject in addition to burying and deserting parasitized eggs. Two out of the three ejected and recovered non-mimetic eggs did not have peck marks, however, the warblers may not have need to puncture the eggs to eject them. Yellow Warblers have been observed removing cowbird eggs with their wings and hollow non-warbler eggs from their nests with their breast and between their legs (M. Kuehn, pers. comm.), but it would be more difficult, however, for warblers to remove cowbird eggs weighing 3 g between their legs. However, cuckoo hosts have rolled parasitic eggs from shallow nest cups (Marchetti 1992), therefore it is possible that warblers rolled blue eggs out of their nests, as some eggs were found on the ground nearby.

Recorded ejections and extended peering also suggest that cowbird eggs mimic warbler eggs. Klippenstine and Sealy (2008, in press) found that non-mimetic blue eggs were ejected more frequently than cowbird eggs in grassland host species, which suggests that similarity between host and cowbird eggs prevented host discrimination of the parasitic egg. Blue eggs of Black-billed (*Coccyzus erythropthalmus*) and Yellow-billed Cuckoos (*C. americanus*) were hypothesized to mimic those of certain host species such as American Robins and Gray Catbirds (Hughes 1997), however clutch manipulations showed that these two hosts, whose own eggs are blue, did not eject ancestral white eggs more frequently than blue eggs (Lorenzana and Sealy 2002).

Once settled on an experimentally parasitized clutch, Yellow Warblers shuffled their bodies more frequently (presumably to contact their developing brood patch with the nest contents) and shifted the eggs in their clutch longer than at control nests (Table 11). This strongly suggests they felt the addition of a foreign egg in their clutch by comparing their clutch volume or other tactile parameters before versus after the manipulation (Rothstein 1982b). Indeed, tactile cues may be even more important than visual cues, because we would expect blue non-mimetic eggs to be rejected even more frequently than mimetic cowbird eggs. Therefore, Yellow Warblers may first visually recognize a difference in their clutch after parasitism, but tactile cues are used to reinforce the stimulation provided by the visual cues and are used to make the final decision to abandon their parasitized clutch.

Re-parasitism: is burial innate or under environmental influence in Yellow Warblers?

Less time remained in the breeding season by the time nests were re-parasitized. It would be expected, therefore, that the rejection frequency at re-parasitized nests would be lower than at nests parasitized for the first time (i.e., earlier in the season). The rejection frequency was lower at re-parasitized nests, but the difference was not statistically significant. This may be due to the fact that, on average, re-parasitism events occurred only five days after the first parasitism, which still gave some warblers enough time in the breeding season to bury again and initiate a third clutch. Indeed, unparasitized females routinely re-nest two to three times a season (S. G. Sealy, pers. comm.). If parasitized a third time, even later in the breeding season, I predict that the rejection frequency would have been closer to zero.

Host defence of brood parasitism has been considered to be largely genetic in the sense there is one or more gene that codes for rejection (Rothstein 1975b, Takasu 1998, Soler *et al.* 1999, Moskát *et al.* 2002). Females that rejected the first time did not reject the second time more often than would be expected in a random sample, therefore it does not seem to be a gene in Yellow Warblers that when expressed, always promotes burial, and when not expressed, does not. Rather than being controlled purely by a gene with no interaction with the environment, burial in Yellow Warblers appears to be affected by environmental cues. Most birds may have the "gene" for rejection but the gene is only expressed under certain environmental conditions, such as time remaining in the breeding season. A bird that rejects the first time will not always reject during subsequent parasitism events, but my results did not determine whether accepters always accept.

Accepters could have had their clutch removed to elicit another clutch to be laid in the same nest and then the new clutch could have been parasitized. Unfortunately, this is not feasible as adult warblers probably will desert nests if all their eggs are removed (Sealy 1992). Another possibility would be to follow banded warblers to their re-nesting site and parasitize their second nest or follow them from year to year.

To demonstrate that female Yellow Warblers maximize their fitness by burying or deserting parasitized nests early in the breeding season but accept cowbird eggs later, I described the impact of burial versus acceptance on reproductive success over the breeding season (Fig. 5). The curve was based on Verhulst *et al.*'s (1995) work on the effect of hatch date on reproductive success and recruitment in Great Tits (*Parus major*) and similar curves have also been reported in other passerines (Mermoz and Reboreda 1998). I modified this curve for Yellow Warblers at Delta Marsh by changing the mean reproductive success to 3.23 fledglings and the cost of parasitism to 0.6 fledgling (Lorenzana and Sealy 1999), mean clutch-initiation period to 36 days (Sealy 1995), time between LD2 at the original nest and LD2 at the superimposed nest to 4.1 ± 0.2 days ($n = 24$; this study) and the time for a nest to be built at a new location and for the second egg to be laid to 8.5 ± 0.2 days ($n = 90$; this study). The precise shape of the curve for Yellow Warblers is not known, although the curve in Fig. 5 is likely a good approximation. Yellow Warblers would maximize their fitness by burying cowbird eggs early in the breeding season. If reproductive success decreases at a greater rate, it would be beneficial for warblers to accept cowbird eggs at the end of the breeding season. An alternative explanation is that few birds reject at the end of the season because young birds tend to nest later and are less experienced with parasites, as I have shown that interactions with
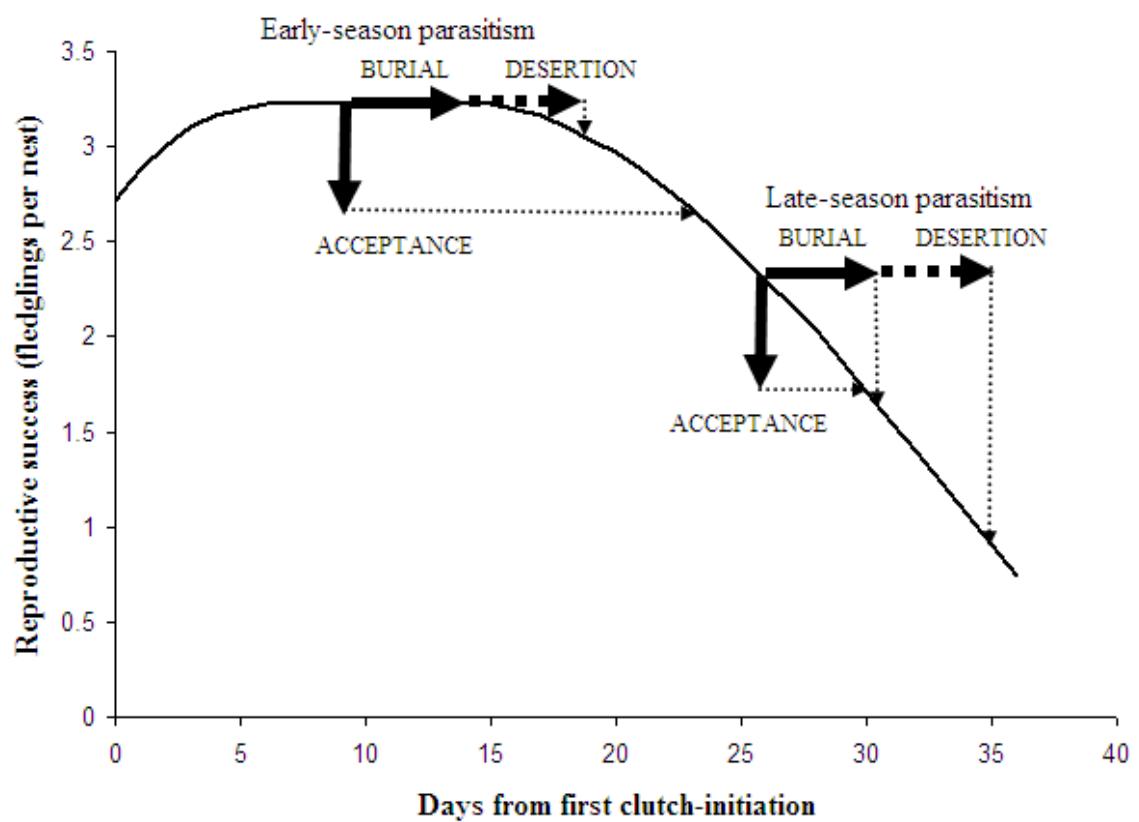
Figure 5. Impact of burial or desertion versus acceptance on reproductive success in Yellow Warblers over the breeding season. A similar curve was reported by Guigueno and Sealy, in press.

the parasite ("experience") may play a strong role in host response. Similarly, few birds may reject at the end of the season because they have had *more* interactions with parasites, and this may lead to fewer rejections ("the Mafia hypothesis"; see above), although I have rejected this hypothesis earlier.

Previous authors have treated most responses as a completely genetic behaviour (Rothstein 1975b, Takasu 1998, Soler *et al.* 1999). Rothstein (1975b), who considered only ejection as a method of rejection, used a model that derives a selection coefficient for acceptance and rejection to calculate how long rejection would become fixed in a population. Rothstein (1975b) calculated that it would take between 20-100 years for ejection to spread through the population of four accepter species. No doubt there is a strong genetic component, even in Yellow Warblers; however, burial and ejection are different in terms of costs because ejection is cost-free if no egg recognition errors are made and host eggs are not removed (Rothstein 1975b; Lorenzana and Sealy 2001).

My work shows that genetic factors must interact with phenotypic plasticity, influenced by environmental cues, and it is this interaction that probably determines the final response. In individuals that have the gene(s) to reject (Rothstein 1975b), the decision to abandon, especially to bury, is the result of interacting cues (Fig. 6). Decision-making is regarded as a cognitive process leading to a choice among alternatives (Schaller *et al.* 2007; Harris 2008). Therefore, individuals that have the genetic make-up to reject, must make a contingent decision (Harris 2008). The decision to reject is already made, but the decision is put on hold until certain cues are presented. If there are no cues, acceptance will be the default decision. Acceptance or rejection has a different value, depending on the situation (Harris 2008), but the goal of this decision is to maximize
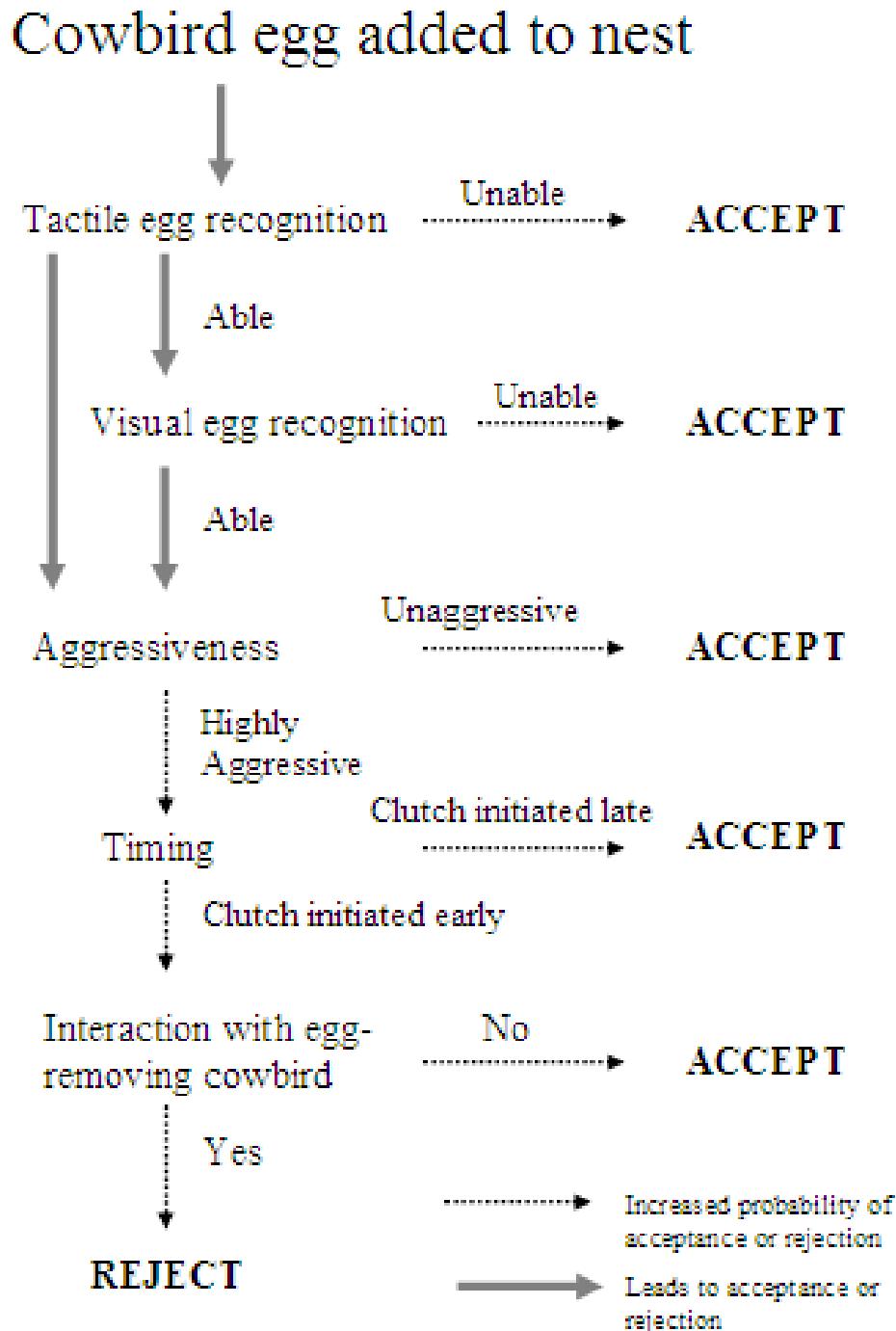
Figure 6. Factors affecting a warbler's decision to accept and reject cowbird eggs. These only include key factors as other factors may be involved.

reproductive fitness (Fig. 5). I have identified three cues that affect the warbler's decision to reject: presence of a parasitic egg in the nest, interaction with an egg-removing cowbird, and time of parasitism relative to progression of the breeding season. My results also have shown that aggressiveness is correlated with rejection. The decision-making process of the Yellow Warbler is presented in Fig. 6. The value of rejection or acceptance depends on the presence of multiple cues. If none of the cues in Fig. 6 is present, especially a parasitic egg in the nest, the value of rejection is low because rejection itself has costs (see above). However, if the parasitic egg is laid early in the breeding season, the value of rejection increases because the cost of acceptance is greater than the cost of rejection (Fig. 5 and 6).

The cost of rejection may be why some hosts of brood parasites have not developed egg rejection (evolutionary equilibrium hypothesis; Rohwer and Spaw 1988, Lorenzana and Sealy 2001). My study has shown how multiple cues act as a stimulus for rejection in a cowbird host (Schaller *et al.* 2007), which contributes importantly to our understanding of avian brood parasitism and decision-making but also of evolutionary ecology because multiple cues have been shown to affect the frequency of an adaptive trait (Price *et al.* 2003, Davies and Welbergen 2009). Changes in egg recognition and rejection affect population dynamics of brood parasites and their hosts (Takasu *et al.* 2003, Davies and Welbergen 2009).

Other factors influencing Yellow Warbler rejection behaviour: future research

Hormone levels vary with age, experience and aggressiveness (Hutchinson *et al.* 1967, Wingfield and Soma 2002, O'Dwyer *et al.* 2006) and testosterone, in particular, may play a critical role in determining host response as I have shown that more

aggressive birds are more likely to reject parasitism. Birds with high levels of prolactin, which is associated with parenting and is stimulated by the "feel" of eggs on the brood patch, may be less willing to reject eggs. Birds with high levels of testosterone, which is associated with aggressiveness, may defend against a parasite and reject eggs if they are parasitized. Birds with high levels of corticosterone, which is associated with stress and promotes increased investment in foraging, may react negatively to an abnormal change in clutch volume or other stressors (e.g., parasitism) and therefore reject eggs. As hormone levels vary with age and experience (Wingfield and Soma 2002, O'Dwyer *et al.* 2006), testing whether egg rejection frequencies are caused by differences in hormone levels will allow researchers to gain insight into the proximate mechanisms underlying these behaviours. Future studies like the one described above would help researchers understand better which physiological factors influence rejection and clutch abandonment in Yellow Warblers and other hosts of brood parasites.

By identifying a physiological basis for egg rejection, we could identify a proximate cause of egg rejection in hosts that abandon parasitized clutches. This will provide a basis for testing the evolutionary equilibrium hypothesis (Rohwer and Spaw 1988), a paradigm for understanding why some hosts accept parasitism. This hypothesis posits that acceptance has been selected because the cost of rejection exceeds the cost of acceptance (Rohwer and Spaw 1988). There are costs associated with high hormone levels, such as reduced survival or reproductive success (Wingfield 2003, Korte *et al.* 2005), and there may be an equilibrium between rejecting eggs (e.g., high cort/T) and improving fitness in the absence of parasitism (low cort/T). Researchers could use the relationship between hormones and reproductive success from field work, coupled with a

range of possible survival outcomes from the literature, to model mathematically the costs and benefits of varying hormone levels. This will directly test the evolutionary equilibrium hypothesis, tie proximate mechanisms with their ultimate causes and provide a robust model for a hormonal basis for host-parasite interactions. This potential future work will therefore bridge the gap between physiology and behaviour in the context of avian brood parasitism, significantly adding to our understanding of host-parasite interactions in the fields of behavioural ecology and evolution.

SUMMARY

1. In 2008, addition of an experimental egg before sunrise elicited the highest rejection frequency among all groups, which was similar to the rejection frequency (31.8%, $n = 22$) at naturally parasitized nests (27%, $n = 11$).

2. The most aggressive individuals were the most likely to bury their parasitized clutches, thus sacrificing their own eggs.

3. Interaction with an egg-removing cowbird model in mid-morning increased the probability of rejection.

4. Presence of a laying model on the nest before sunrise decreased the probability of rejection, likely because the model was not moving like a live cowbird.

5. In 2009, model cowbird eggs added to nests before and after sunrise were rejected at similar frequencies (29.7%, $n = 74$ versus 22.9%, $n = 70$, respectively).

6. Non-mimetic blue eggs (31.1%, $n = 74$) were not rejected significantly more frequently than cowbird eggs (21.4%, $n = 70$), although only blue eggs were ejected from nests.

7. All experimental nests in 2009 that received any egg at any time elicited a rejection frequency (26.4%, $n = 144$), similar to the rejection frequency recorded at nests naturally parasitized during laying (20.0%, $n = 5$).

8. Yellow Warblers returning to nests after egg addition peered significantly longer at their clutch than at control nests. Individuals that received an egg shuffled their bodies more frequently and spent more time probing eggs with their bill once settled on their parasitized clutch.

9. The rejection frequency at re-parasitized nests (5.0%, $n = 19$) was smaller, but was not significantly different from the rejection frequency at nests parasitized for the first time (21.4%, $n = 135$).

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APPENDIX

Upon examining the distribution of experimental nests, I noticed that rejected clutches seemed clumped (Fig. A.1, A.2, A.3, and A.4). The average distances between rejected clutches seemed smaller than expected given the average distance between all experimental clutches. I used the randomization test to analyze the nearest-neighbour distance between a group of randomly chosen experimental nests (Clark and Evans 1954, Donnelly 1978). Using Visual Basic Macros in Excel 2003, I created a computer model that calculated the average nearest neighbour distance between all rejected clutches in 2008 and 2009. A z-test in Excel was then used to compare the average nearest-neighbour distance between rejected clutches to the average nearest neighbour distances of the thirty simulations of randomly chosen experimental nests.

I divided the field site into three sections to take into account possible edge effects between different sections of the field site: Cram Creek to the west side of the diversion (Site 1), east side of the diversion to the cottage area (Site 2), and the Bell estate (Site 3) (Figure A.1). Sites 2 and 3 were separated by a couple of nests, which were omitted from the analyses. Analyses for the 2009 data are more reliable than the analyses for the 2008 data because four treatments in 2009 elicited rejection at a frequency higher than 20%, compared to only one group in 2008 (Tables 6 and 9). Therefore the sample sizes of rejected clutches were greater in 2009. Rejected clutches that are (1) closer to each other than to randomly chosen experimental nests are "clumped," (2) farther from each other than to randomly chosen experimental nests are "uniformly" distributed, and (3) at a similar distance to each other than to randomly chosen experimental nests are "randomly" distributed.
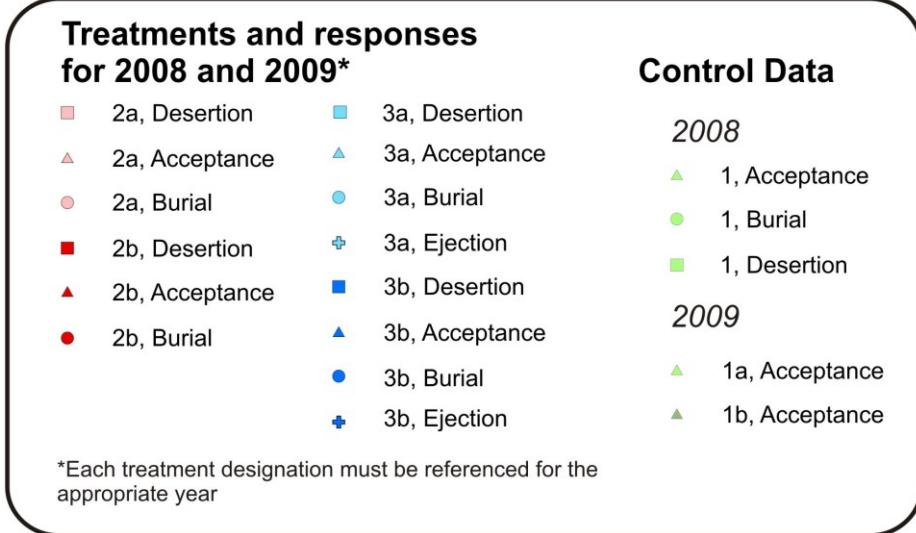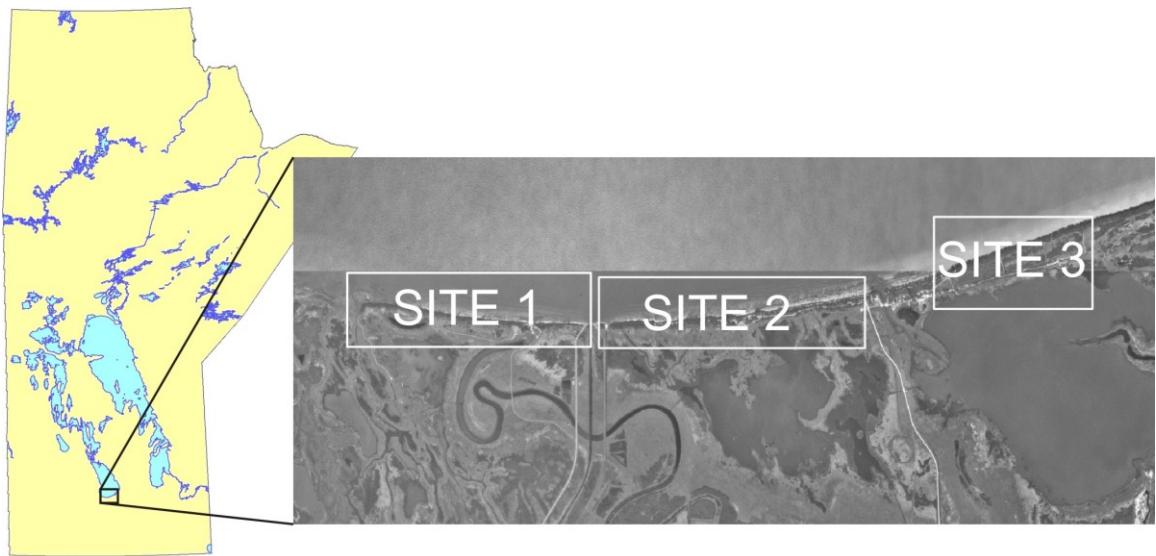
Figure A.1 Legend for the distribution of nests from Experiment 1 (2008) and Experiment 2 (2009).
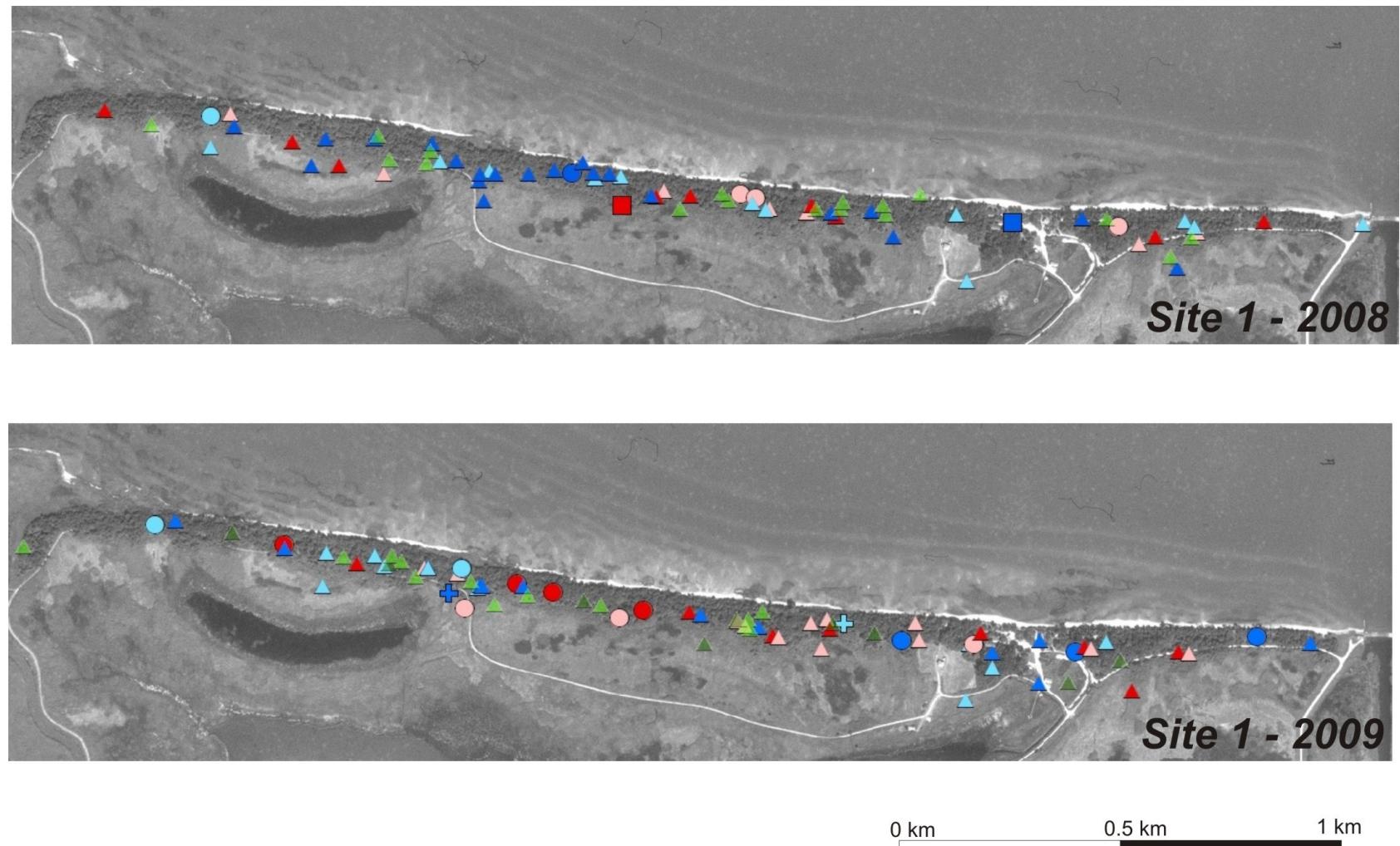
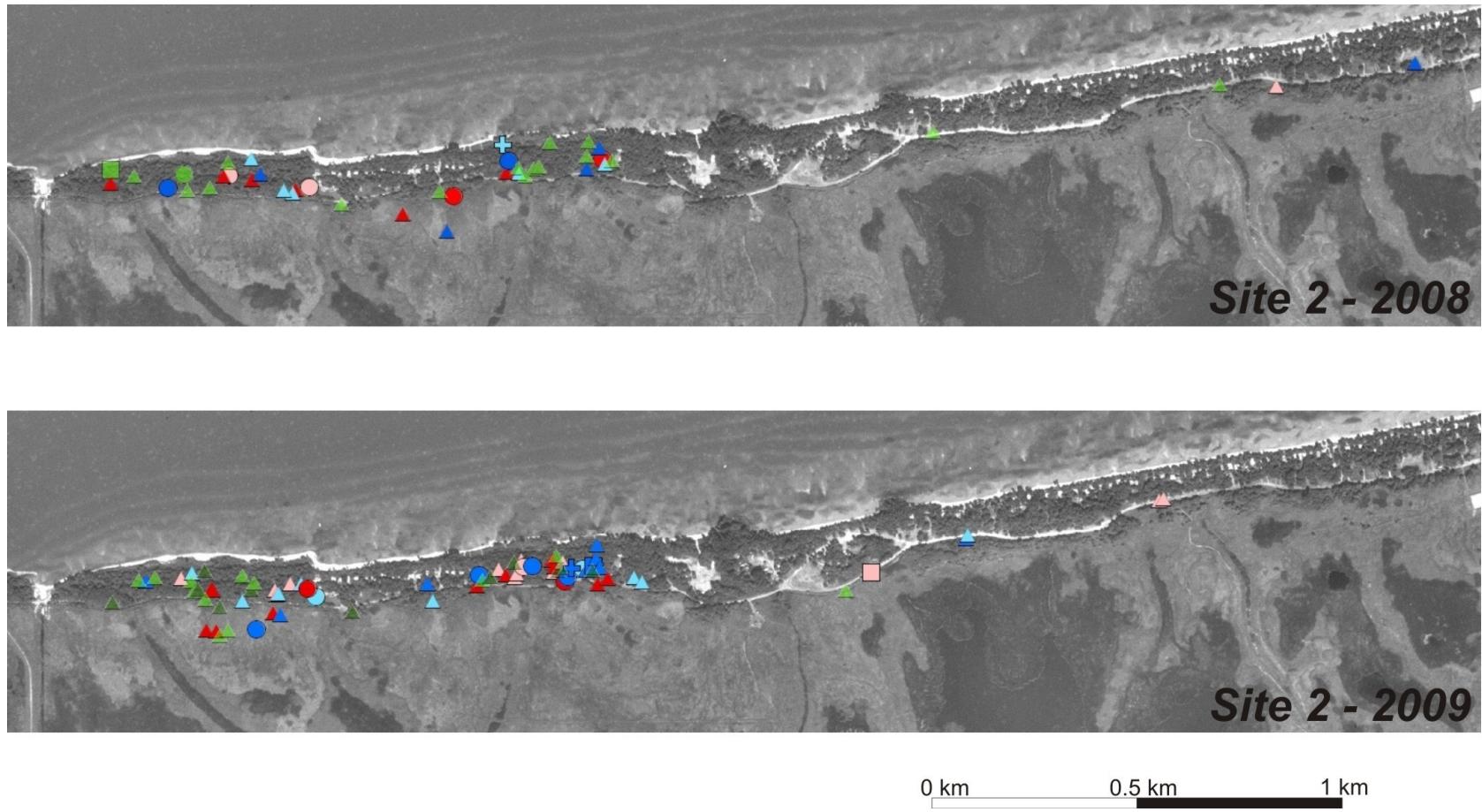**Figure A.2** Distribution of experimental nests on the field station property (Site 1).

Figure A.3 Distribution of experimental nests on the east diversion section (Site 2).
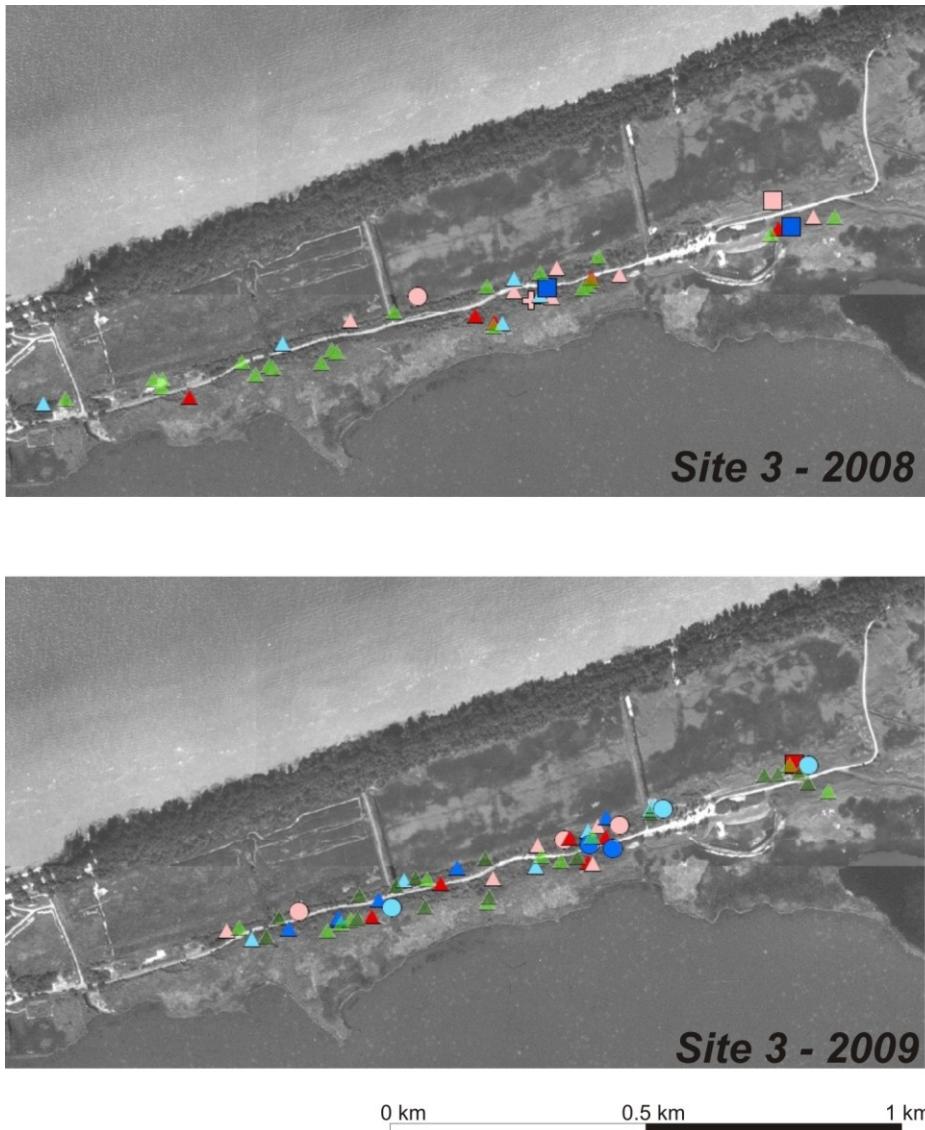
Figure A.4 Distribution of experimental nests on the Bell Estate (Site 3).

The most common distribution pattern was "clumped" at 50% for both years and at 66% in 2009, when samples sizes of rejected clutches were larger (Table A.1). Non-random clumping of rejected clutches could be due to several factors that should be investigated further. Clumping was recorded in 2008 and 2009 in Site 3. In this area, most nests are located at the edge of the marsh and forest ridge (M. F. Guigueno, pers. obs.). Therefore, nests would be more easily seen from a tree in the ridge in Site 3 than in the other two sites where nests are located throughout the ridge. Nests are more likely to be parasitized by female Brown-headed Cowbirds if they are near trees, as they scan for host nests from trees (Clotfelter 1998). It is possible that areas with high rejection frequency were also areas with high cowbird activity (due to good perching trees) through host egg-removal visits and cowbirds searching for nests (Sealy 1992). Thus, increased interaction with the parasite in addition to experimental addition of a cowbird egg would increase the probability of rejection (Moksnes and Røskaft 1989, this study), especially if this interaction occurs during the day (i.e., after sunrise; see Results). Cowbirds may visit Yellow Warbler nests to remove a host egg without parasitizing nests because, over multiple breeding seasons at Delta Marsh, single host eggs have disappeared from some nests that were monitored daily without subsequent parasitism (D. Campobello, M. F. Guigueno, S. G. Sealy, pers. obs.). To support this hypothesis, it would be necessary to track individual cowbirds using passive inductive transponder (PIT) tags to determine which nests are visited and thus which hosts interacted with cowbirds the most. Clumping was also recorded on Site 2 in 2009 and this is consistent with the possibility that clumped rejected clutches were due to high cowbird activity because naturally parasitized nests from both years seemed clumped in Site 2 (Fig. A.5).

Table A.1 Average distance (m) between rejected clutches and clutches chosen at
random and distributional pattern in the three areas of the field site in 2008 and 2009.

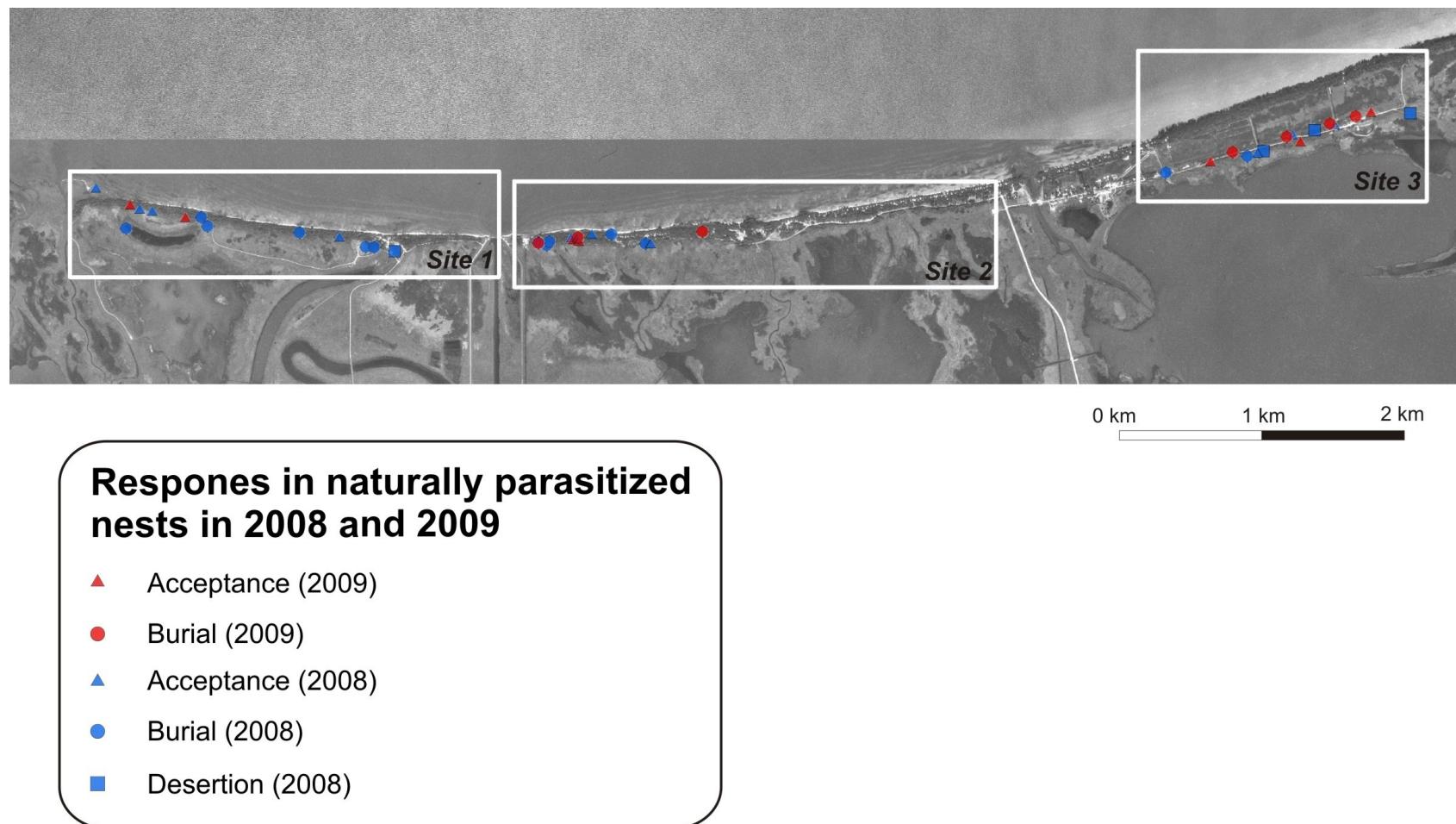|	Distance between rejected clutches (*n*)	Distance between random clutches (±SE)	*z*-value	*p*-value	Distribution
Site 1					
2008	228 (7)	219 ± 13	0.75	0.46	Random
2009	145 (14)	111 ± 5	6.66	<0.01	Uniform
Site 2					
2008	130 (8)	85 ± 5	8.53	<0.01	Uniform
2009	84 (13)	100 ± 6	2.59	0.01	Clumped
Site 3					
2008	176 (4)	260 ± 17	14.91	<0.01	Clumped
2009	77 (11)	88 ± 3	3.32	<0.01	Clumped

Figure A.5. Distribution of naturally parasitized nests in 2008 and 2009.

Another possible cause for clumping of rejected clutches is that more experienced individuals, which have learned to recognize they have been parasitized and subsequently reject a parasitized clutch, nest more closely together in higher quality habitat. Sealy (1995) found no differences in rejection frequency between younger and older individuals, but age does not necessarily measure the level of experience, especially if older individuals were not parasitized in previous years. However, older individuals may select higher quality habitat. In past years, when willows (*Salix interior*) were more abundant along the southern edge of the riparian forest, after second-year adults were observed selecting nest sites in the southern part, whereas second-year adults that arrived at the breeding site later, selected nest sites in the northern part (MacKenzie 1982; S. G. Sealy, pers. comm.). However, the characteristics of the riparian forest at Delta Marsh has changed since two decades ago and now there are few willows and Yellow Warblers nest in elderberry (*Sambucus* spp.) bushes, which are more numerous in the northern part of the riparian forest (M. F. Guigueno, pers. obs.). To test the hypothesis that host experience and habitat quality play a role in clumping of rejected clutches, it would be necessary to follow individual warblers over multiple years, measure their nest-site characteristics, and record whether they are naturally parasitized and their response to parasitism (acceptance or rejection). Although experimental nests in 2008 and 2009 were frequently clumped, more investigation is needed before we understand why this occurs or even whether this is representative of most years.