Western Graduate&PostdoctoralStudies

Western University
Scholarship@Western

Electronic Thesis and Dissertation Repository

3-28-2018 2:00 PM

Nest Predation by brown-headed cowbirds (Molothrus ater)

David C. Swan, *The University of Western Ontario*

Supervisor: Zanette, Liana Y., *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biology

© David C. Swan 2018

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Biology Commons

Recommended Citation

Swan, David C., "Nest Predation by brown-headed cowbirds (Molothrus ater)" (2018). *Electronic Thesis and Dissertation Repository*. 5274.
https://ir.lib.uwo.ca/etd/5274

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlswadmin@uwo.ca.

Abstract

The reproductive success of parasites is entirely dependent on their ability to encounter suitable hosts. Obligate brood parasitic birds may increase host encounter rate, and consequently their reproductive output, if they cause unsuitable late-stage host nests to fail thereby stimulating the host to create another nest that they can parasitize. I tested key predictions of this 'farming' hypothesis for the brown-headed cowbird (*Molothrus ater*). I found evidence that cowbird attacks are not uncommon, a basic requirement of the hypothesis. Furthermore, I found multiple lines of evidence that cowbird attacks are not indiscriminate, but directed at non-parasitized nests and at those at a developmental stage too late to be suitable for parasitism. I experimentally demonstrate that cowbirds determine the age of a nest by directly puncturing a portion of the clutch or indirectly by attending to the absolute number of eggs. Cowbirds also parasitized a high proportion of the re-nesting attempts following their attacks suggesting that they take advantage of the reproductive opportunities they create. A Monte Carlo model comparing simulated farming and non-farming cowbirds also shows that a farming strategy may lead to higher reproductive output likely by enhancing individual nest discovery as opposed to increasing the number of nests. How cowbirds occupy space may also provide insight into how they encounter potential hosts. Utilization distributions (UDs) are among the most applicable methods of quantifying space use. In one of the first practical applications of a multidimensional UD that includes time as a dimension, I show that cowbirds were significantly more likely to be found around nesting sites when a nest was active suggesting that cowbirds optimize their nest searching. I did not, however, find a difference in probability of occurrence depending on the developmental stage of a nest. I also found evidence that cowbirds become less territorial later in the day. Comparisons to strictly spatial UDs suggest that including a time dimension may

provide a more realistic model of how cowbirds find host nests and interact with one another. Thus, cowbirds can discriminate appropriate vs inappropriate nests and adjust their predatory and spatial behaviour accordingly to improve their encounters with hosts.

Keywords: brood parasitism, brown-headed cowbird, *Molothrus ater*, farming hypothesis, home range, host manipulation, host-parasite evolution, infanticide, territory, utilization distribution.

Co-Authorship Statement

I wrote Chapter one of this Thesis and it is not published.

Chapter 2 has not yet been published. I am in the process of writing a version of this chapter that will be submitted for publication. Michael Clinchy will be second author and Liana Zanette will be third author for this submission. Dr. Zanette and Dr. Clinchy funded the project, provided field data from years prior to 2009, contributed to the design of the study, gave advice on statistical analysis and provided editorial comments on the manuscript. Dr. Paul Furgale co-wrote the computer code in Python for the Monte Carlo simulations described in this Chapter, but has asked to not be included as an author on any publications for professional reasons.

A version of Chapter 3 was published: Swan DC, Zanette LY and Clinchy M. 2015. Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis. *Animal Behaviour* 105:29-35. Dr. Zanette and Dr. Clinchy funded the project, provided field data from years prior to 2009, contributed to the design of the study, gave advice on statistical analysis and provided editorial comments on the manuscript.

Chapter 4 has not yet been published. I am in the process of writing a version of this chapter that will be submitted for publication. Michael Clinchy will be second author and Liana Zanette will be third author for this submission. Dr. Zanette and Dr. Clinchy funded the project, contributed to the design of the study, gave advice on statistical analysis and provided valuable editorial comments on the manuscript.

I wrote Chapter five of this Thesis and it is not published.

Acknowledgements

First and foremost, I thank my two supervisors, Dr. Liana Zanette and Dr. Michael Clinchy for all of their support and guidance over the course of my degree. I owe much of my ability as a researcher, communicator and scientist to Liana and Mike. I cannot overstate my gratitude for their patience and their dedication to me and my academic career.

Thanks to the members of my advisory committee, Dr. Bryan Neff and Dr. David Sherry. Dr. Neff and Dr. Sherry helped greatly in refining my Thesis topic, interpreting results and have challenged me greatly during each of our meetings. Thanks in particular to Dr. Neff for providing feedback on an earlier version of this Thesis.

I thank all of my 'Zanchy' lab mates over the years. Not only have they provided valuable feedback on my work, but have been sources of support, partners in commiseration and true friends. I thank Marek Allen, Michelle Bondy, Angela Freeman, Amy Medue, Daniele Ouellette, Sarah Tancredi, Doris Tang and Asia White for their valuable assistance with data collection in the field. A special thanks to Beryl Clinchy for being a gracious host to me and my assistants while in the field. Thanks to Rithet's Bog Conservation Society, Swan Lake Nature Reserve and the Saanich Municipality for access to study locations. Thanks also to the Long Point Bird Observatory and the Ruthven Park Bird Banding Station for providing the cowbirds used in Chapter 2. Thanks to Dr. Paul Furgale for all of his help with my simulation model in Chapter 3.

My research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canadian Graduate Scholarship, the Society of Canadian Ornithologists Baillie Award, the American Museum of Natural History Chapman Research Grant, and a University of Western Ontario Graduate Thesis Research Award.

Lastly, I am extremely thankful for my family for all of their unending love and support. I am especially thankful for my wife, Maria. I am grateful for her selflessness and understanding during my entire academic carrier. The stress of a Ph.D. coupled with long periods away from one another has been difficult at times. Whether she is teaching by example in her own school and work-life or by just giving be a kick in the butt when I need it, Maria has been my principal source of motivation and inspiration.

Table of Contents

Abstract……i
Co-Authorship Statement……...iii
Acknowledgements……iv
List of Figures……ix
List of Appendices……xv
Chapter 1……..…1
1 General Introduction……1
1.1 Parasitism as a lifestyle……1
1.2 Host behavioural manipulation by parasites……..…...2
1.3 Infanticide and behavioural manipulation……...…..6
1.4 Social and brood parasitism……..7
1.5 Avian brood parasitism……...10
1.6 Animal home ranges and home range estimators……...13
1.7 Study species: Brown-headed Cowbird (*Molothrus ater*) ……16
1.8 References……...18
Chapter 2……32
2 Interspecific infanticide by the brown-headed cowbird: an evaluation of the farming hypothesis……..……33
2.1 Introduction……33
2.2 Methods……38
2.2.1 Study site and field methods……38
2.2.2 Predator identification key……..40
2.2.3. Statistical Analyses……41
2.2.4. Simulation model……42
2.3 Results……47
2.4 Discussion……53
2.5 References……58
Chapter 3……68

3 Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis……..68
3.1 Introduction…….……68
3.2 Methods……72
3.2.1 Experimental Procedures……72
3.2.2 Attacks and Developmental Stage……...74
3.2.3 Attacks and Egg Number……75
3.2.4. Attacks and Changing Number of Eggs……77
3.2.5. Field Procedures……79
3.2.6 Statistical Analyses……..79
3.3 Results……..80
3.3.1 Attacks and Developmental Stage……...80
3.3.2 Attacks and Egg Number……82
3.3.3 Attacks and changing number of eggs……83
3.3.4 Wild Cowbird Predation……..84
3.4 Discussion……87
3.5 References……91
Chapter 4……97
4 Modelling breeding ranges in space and time: cowbirds follow available nests and avoid each other……...97
4.1 Introduction……97
4.2 Methods ……102
4.2.1 Study site……102
4.2.2 Capture and radio-tracking……103
4.2.3 Parentage analysis using microsatellite DNA loci……..…105
4.2.4 Creating Utilization Distributions……..106
4.2.5 Estimating relative activity around nests over time……...107
4.2.6 Overlap among cowbird breeding ranges …… ……109
4.2.7 Statistical analyses……112
4.3 Results……...113
4.3.1 Relative activity around nests over time……113

4.3.2 Overlap among cowbird breeding ranges: two-versus three-dimensions……..115
4.3.3 Daily patterns in group size and aggression……..117
4.3.4 Egg-laying areas……119
4.4 Discussion……121
4.5 References……127
Chapter 5……134
5 General Discussion……...134
5.1 Summary……134
5.2 Cowbirds manipulate their hosts……136
5.3 Specific challenges for studying cowbird predatory behaviour……136
5.4 Implications for future home range studies……...138
5.5 Implications for conservation……139
5.6 Future directions……140
5.7 Conclusions……142
5.8 References……..144
Appendix 1: Animal use protocol approval for Chapter 3……...149
Appendix 2: Python code for Monte Carlo simulation in Chapter 2……...150
Appendix 2.1 Create the 'cowbird' object……..150
Appendix 2.2 Create the 'nest' object……151
Appendix 2.3 Running a single simulation……154
Appendix 2.4 Run experiment using multiple simulations for multiple cowbird types…….157
Appendix 3: Actual values from Monte Carlo simulation in Chapter 2……..161
Curriculum Vitae……164

List of Figures

Figure 2.1: Mayfield's daily survival rates for parasitized and non-parasitized nests. Error bars indicate Standard Deviation. …….……49

Figure 2.2: The proportion of nests attacked by cowbirds and non-cowbirds that were parasitized at the time of attack. Numbers inside bars indicate sample size. Error bars indicate Standard Error. ……...49

Figure 2.3: The proportion of nests attacked by cowbirds and non-cowbirds that were parasitized at the time of attack. Numbers inside bars indicate sample size. Error bars indicate Standard Error. ……..……50

Figure 2.4: The proportion of cowbird chicks fledged by simple (blue) and advanced farmers (red) to that of non-farming cowbirds. Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*). Values above the dotted line are greater than that of non-farmers. ……..……..52

Figure 3.1: The proportion of eggs punctured or removed from experimental nests containing developed late-incubation eggs and nests containing undeveloped early-incubation stage eggs. Error bars indicate Standard Error. ……..……..81

Figure 3.2: The proportion of eggs punctured or removed from experimental nests containing developed late-incubation eggs and nests containing (a) four-egg and two-egg

clutches and (b) six-egg and four-egg clutches. Error bars indicate Standard Error……..……..83

Figure 3.3: The proportion of eggs killed by cowbirds in non-parasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest (N = 74). Points are offset slightly where overlap occurs. …….85

Figure 3.4: The proportion of eggs killed by cowbirds in parasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest (N = 64). Points are offset slightly where overlap occurs. ……..86

Figure 4.1: Cowbird home range and host nests visualized across time using adehabitat (Calenge 2006). The outer line in each panel represents the home range of a single bird estimated at different days. The numbers represent the locations of four song sparrow nests. The magnitude of the numbers indicate the age of the nest relative to the first day of incubation… … …… … … ……. …108

Figure 4.2: Overlapping 95% utilization distributions of two neighbouring brown-headed cowbird females in two-spatial dimensions (left panel) and three space and time dimensions (right panel). Easting and northing are UTM coordinates in meters……111

Figure 4.3: Relative activity of cowbird females (standardized height of utilization distributions) around nests across different stages of development……...114

Figure 4.4: Comparison of two-dimensional spatial and three-dimensional spatio-temporal estimates of home range overlap among female cowbirds. Points above the line indicate overestimation of overlap using two-dimensional spatial methods. ……116

Figure 4.5: Number of male and female cowbirds (mean ± SE) observed with radio-tracked females throughout the day. ……118

Figure 4.6: Proportion of aggressive interactions among female cowbirds throughout the day. ……...……118

Figure 4.7: Home ranges and locations of host nests parasitized by female cowbirds in 2009. Each colour represents a different cowbird. Solid lines represent home range boundaries defined by the 95% isopleth of two dimensional utilization distributions. Triangles represent the location of specific parasitized nests. Triangles multiple colours indicate multiple parasitism. Egg laying areas for females laying eggs in at least three different nests are shown as minimum convex polygons (dashed lines) encompassing the locations of parasitized nests. Single dashed lines connect two nests parasitized by the same female. Easting and northing are UTM coordinates in meters……120

Figure App3.1: The number of cowbird eggs laid by non-farmers (grey), simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*). ……161

Figure App3.2: The number of cowbird chicks fledged by non-farmers (grey), simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*). ……162

Figure App3.3: The number of attacks on host nests by simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*). ……163

List of Appendices

Appendix 1: Animal use protocol approval for Chapter 3…….149

Appendix 2: Python code for Monte Carlo simulation in Chapter 2 …….150

Appendix 3: Actual values from Monte Carlo simulation in Chapter 2. …….160

Chapter 1

1 General Introduction

My thesis has two main objectives. First, I examine how infanticidal behaviour of the brood parasitic brown-headed cowbird (*Molothrus ater*) may improve their reproduction (*sensu* the farming hypothesis). This topic is addressed within the larger context of how parasites may improve their transmission by manipulating the behaviour of their hosts (Chapter 2 and 3). Second, I employ recently developed techniques in spatial analyses to examine how available nests and social interactions influence cowbird space use (Chapter 4). In this general introduction, I first present an overview of the importance of parasitism as a lifestyle followed by a comprehensive summary of parasite-mediated manipulation of host behaviour. I then provide a review of how infanticide may also serve as a strategy to increase reproduction, specifically drawing parallels to behavioural manipulation by parasites. Next, I present a literature review on the social and brood parasites. I then review brood parasitism specifically in birds and highlight the diversity of strategies involved, the impact on host fitness, and examples of how avian brood parasites likely manipulate host behaviour. I provide a synopsis of historic and contemporary space use and home range analyses. Finally, I present a brief overview of the relevant biology of the brown-headed cowbird.

1.1 Parasitism as a lifestyle

Parasitism likely constitutes the most common lifestyle on the planet (Toft 1986; Thompson 1994) and interactions among parasites and 'free-living' organisms are both diverse and complex (Moore 2002). Perhaps because parasites constitute only a small fraction of the biomass within a

community, they have historically been considered to be of minor ecological importance (Poulin 1999; Lefevre et al. 2008). In the last few decades, however, ecologists have begun to recognize the influence parasites exert on not just individual hosts, but population dynamics, community structure and biodiversity (Thompson 1999; Wood et al. 2007). Given the ubiquity of parasites, understanding their role in ecological communities has become a central question in ecology, evolution and conservation biology (Rozsa 1992; Thomas et al. 2005). Typically, parasites negatively impact the fitness of their hosts (Price 1980) by increasing mortality, reducing fecundity or lowering competitive ability. The reproductive fitness of any parasite depends on its ability to successfully infect an individual host, replicate within and then disperse to other hosts. Absolutely key to this process is the likelihood that a parasite, while in its infective stage, will encounter susceptible hosts. Metaphors such as an 'arms race' (Dawkins and Krebs 1979) and 'Red Queen' (Van Valen 1973) are often evoked to explain the ceaseless process of reciprocal coadaptation among antagonistic parasites and their hosts while either side seeks to change the probability of these encounters.

1.2 Host behavioural manipulation by parasites

Parasites exhibit a vast array of strategies to enhance the transmission of themselves or their offspring, by exploiting typically heterospecific hosts (Poulin 2007; Schmid-Hempel 2011). One particularly fascinating example occurs when parasites manipulate the behaviour of their hosts in ways that facilitate their own transmission (manipulation hypothesis: Holmes and Bethel 1972, Poulin 2000). In such cases the modified behaviour can be thought of not being due to the expression of the host's genes, but instead may be considered an extended phenotype of the parasite itself (Dawkins 1982; Hughes et al. 2012). Parasite induced behavioural change may

have direct consequences for conservation, ecology, medicine, agriculture and evolutionary trajectories and, as such, has generated an appreciable amount of theoretical and empirical research (Moore 2002; Lafferty 2006, Flegr J. 2007. Reiber et al. 2010).

Few animals infected by a parasite or pathogen behave just like uninfected animals and changes in behaviour can vary greatly in extent, from modest changes in time spent performing an ordinary activity, to the emergence of radical new and bizarre behaviours (Moore 2002; Poulin 1994). For example, sandflies (*Phlebotomus spp.*) infected with the protozoan *Leishmania sp.* moderately increase their biting frequency (Rogers and Bates 2007). At the opposite extreme, ants infected by the tremetode "brain worm" *Dicrocolelium dendriticum* uncharacteristically leave the protection of their colony, ascend a blade of grass and remain there clamped down with their mandibles (Carney 1969; Wickler 1976). In both these examples, the behaviour modification would appear to increase the probability that the parasite will encounter other hosts. *Leishmania* is transmitted to its mammalian definitive hosts through the bites of sandflies and ants atop a blade of grass would presumably be more likely to be eaten by the definitive sheep host of *Dicrocolelium sp.* The consequences of these examples appear fairly clear because of the obvious connection to the parasitic lifecycle, however, the adaptive significance of parasite-mediated behavioural change may not be so easy to ascertain (Dantzer 2001, Adamo 2013).

The vast majority of studies on parasite-mediated behavioural change have merely been reports on the phenomenon itself. Typically, unusual or aberrant behaviours are observed in infected individuals and, if this seems as though it could increase transmission then the behavioural change is assumed to be an adaptive strategy on the part of the parasite (Beckage 1997; Moore 2002). While this practice is a valuable first step in identifying possible examples of the manipulation hypothesis, it has been criticized as adaptationist story telling (Gould and

Lewontin 1979; Poulin 2000). It has only been relatively recently that researchers have emphasized that alternative explanations need to be considered before accepting adaptive manipulation (Klein 2005; Thomas et al. 2005; James 2010; Cator et al 2012, 2014; Lafferty and Kuris 2012; Worth et al. 2013). Behavioural change following infection may simply be a side-effect or even a maladaptation manifested because of some other trait (Heil 2016). Alternatively, a behavioural change may represent a host adaptation aimed to reduce or compensate for the detrimental consequences of infection (Lefevre et al. 2008). Perhaps sandflies combat infection through increased nutritional uptake or brainworm-infected ants leave to distance themselves from their uninfected relatives. In either example, this is likely not the case (Rogers and Bates 2007; Highes et al. 2012), but I suggest hypothetical alternative explanations as a way to highlight the need for additional research before drawing such a conclusion.

To understand the connection between infection and host behavioural change it is absolutely necessary to consider both the adaptive function and the proximate mechanisms involved (Klein 2003). Even in cases where intuition suggests that host behaviour changes benefit the parasite, I suggest three criteria must be satisfied before drawing such a conclusion. Firstly, it is necessary to establish that the behavioural change is actually caused by the parasite (Moore 2002). Secondly, the behavioural change must be shown to increase the probability of transmission— often determined by comparing the probability that infected versus uninfected individuals encounter additional hosts (Lafferty 1999; Berdoy et al. 2000). Thirdly, it is critical to identify the specific mechanisms involved in the behavioural change in order to understand the complexity and sophistication of the supposed manipulation (Nickol 2005; Thomas et al. 2005). Throughout this thesis, these are the three stringent criteria I return to when considering whether manipulation of a host is an adaptive parasitic strategy.

1.3 Infanticide and behavioural manipulation

Infanticide among animals is a widespread occurrence with no single explanation (reviewed in Chapter 2). Predominant among these is the hypothesis that infanticide is a male strategy that enhances their reproductive success at the expense of the female (sexual selection: Hrdy 1974). Essentially, newly arrived males kill the unrelated infants of their competitors so that females come into estrous earlier than they otherwise would and rear the intruder's young. Functionally, infanticidal males of several species, especially within mammals, act much like parasites in this way (Palombit 2015). In the broadest possible definition (Esch and Fernandez 1993), parasitism represents a non-mutual relationship between two groups where one benefits (the parasite) at the expense of the other (the host). In this sense, male lions, for example, are a type of parasite. After all, their days are spent predominantly sleeping, impregnating females, stealing their food and contributing next to nothing in care for their own young (Schaller 1972). Although I may be a bit tongue and cheek making the general comparison, the specific parallel to the parasite manipulation hypothesis is striking. Infanticidal males (the parasite) manipulate females (the host) to care for their young at the expense of the female.

Sexually selected infanticide and parasite-mediated behavioural manipulation should be considered within very similar context. As with parasitism, infanticide can represent a considerable source of (juvenile) mortality (Sherman 1981; Packer and Pusey 1983). In response, just as with parasitic hosts, females have evolved a suite of strategies to reduce the incidence of infanticide including cooperative defense of young, provisional absence from a social group, abortion, and delayed or concealed ovulation following the arrival of new males (Hdry 1979; Packer and Pusey 1983; Wolff and Macdonald 2004; Palombit 2015). Just as is needed with

parasite mediated manipulation, I propose that additional hypotheses must be considered and systematically rejected before one can conclude that infanticide represents a behavioural adaptation that facilitates the 'transmission' of the male aggressor.

1.4 Social and brood parasitism

Darwin (1859) suggested that any instinct cannot have been produced for the good of other individuals, but individuals may take advantage of the instincts of others. In a similar assertion, Dawkins (1999) suggested that "any nervous system can be subverted if treated in the right way". Organisms often behave in ways that seem to benefit others rather than themselves and may be explained by either kin selection (Hamilton 1964) or reciprocal altruism (Trivers 1971). A third possibility is social parasitism— an intriguing phenomenon in which a parasite exploits the labour of its hosts without necessarily improving the inclusive fitness or reciprocating actions for the host (Moore 2002). Although social parasites do not necessarily take advantage of host physiology, they are rightfully called parasites in that they capitalize on the social structure of their hosts, exerting an appreciable fitness cost while benefiting themselves (e.g. Mori et al. 2000; Foitzik et al. 2001; Fischer-Blass et al. 2006). By redirecting host labour they divert resources to themselves, presumably reducing their host's reproductive output if not eliminating it altogether (Hölldobler and Wilson 1990; Ortega 1998; Davies 2000).

Social parasitism occurs within and between species, has been documented across a wide range of taxa, and can encompass a range of specialization from the relatively mundane to the utterly remarkable. Undoubtedly, the range of specialization in social parasitism is best exemplified within the Hymenoptera, particularly the ants (Hölldobler and Wilson 1990) and is worth illustrating here. Xenobiotic or 'Guest' ants live within the colony of another species,

freely intermingle with host workers, and may even solicit food from their hosts (Buschinger 1976). Temporary ant parasites are dependent on a host species for colony foundation but can otherwise care for themselves (Buschinger et al. 1990; Topoff and Zimmerli 1993). Inquiline ants are also reliant on other species for colony foundation, but are permanent parasites having lost the ability to care for themselves (Hölldobler and Wilson 1990). Some species have become so well adapted to their parasitic lifestyle that they have lost the worker caste and developed morphological, physiological and behavioural adaptations that promote complete dependence on their hosts (Wilson 1971). The dulotic or 'slave-making' ants push social parasitism to this extreme. Slave-makers practice non-independent colony foundation similar to that observed in temporary parasites (Buschinger 1986), but go further by raiding the nests of neighbouring species to supplement their host worker force (Alloway 1979). Several dulotic ants have become so specialized that they are completely reliant on their hosts for feeding and brood care (Wilson 1975; Stuart and Alloway 1985).

'Brood Parasitism' is one type of social parasitism that involves a parasitic individual leaving its eggs with another who raises the parasitic offspring at the expense of its own (Lack 1968; Rothstein 1990). This type of 'egg dumping' may be facultative and opportunistic. For example, Lace bugs (*Gargaphia spp.*: Tallamy 1985) and some waterfowl species (Ortega 1998) commonly lay their own clutch of eggs among those of an unrelated conspecific. Many other brood parasites are obliged to leave their eggs with other species. Obligate brood parasitism is well known among birds (Ortega 1998), but has also been documented in some fish (Sato 1986; Wisenden 1999) and arthropods (Boulton and Polis 2002). In every case, if the foster parent is unable to differentiate between the 'dumped' eggs and their own, they will incur the costs of caring for extra young (Petrie and Møller 1991).

To successfully infiltrate the social order of others, a social parasite must utilize inherent sensory preferences of their host. This can be accomplished if the parasite can adequately mimic their host's sensory cues necessary for recognition (Howard et al. 2001). The termitophile *Trichopsenius frosti*, for example, biosynthesizes the same chemical signature as its termite host *Reticulitermes flavipes* (Howard et al. 1980). Many avian brood parasites also lay mimetic eggs resembling those of their hosts (Brook and Davies 1998; Moksnes and Røskaft 1995; Gibbs et al. 2000; Stoddard and Stevens 2010, 2011). Additionally, supernormal stimuli may release exaggerated levels of host response. The relatively large size and vocal mimicry of cuckoo chicks persuade their warbler host to deliver increased quantities of food (Davies et al. 1998). Similarly, larvae of the Vespid social parasite *Polistes sulcifer* are able to solicit more food from workers of their host *P. dominulus* than host larvae can (Cervo et al. 2004).

Several theories have been advanced to explain why host species have not ultimately evolved means of rejecting their social parasites. Social parasites may represent a relatively new selection pressure and hosts have not had the time necessary to evolve an appropriate defense. This may happen when a social parasite is introduced into a new geographical location (Brooke et al. 1998; D'Ettorre et al. 2004) or where parasites evolve to switch host species (Fischer-Blass et al. 2006). Parasite-host relationships may then be locked in an evolutionary arms race (Dawkins and Krebs 1979) or a 'Red Queen' scenario (Van Valen 1973) in which the parasite is currently ahead (Foitzik et al. 2003; Fischer and Foitzik 2004). Some have proposed that parasites simply have too little impact for their hosts to evolve means of recognizing them as enemies (Gladstone 1981). Like physiological parasites and pathogens social parasites may have evolved to have less impact on their hosts (Brooker and Brooker 1996; Hare and Alloway 2001). More likely, however, host species have reached an evolutionary equilibrium in the stringency of

their recognition mechanisms (Maynard Smith 1982). That is, the costs of accepting a social parasite are on average outweighed by the possibility of rejecting colony members.

1.5 Avian brood parasitism

Avian brood parasitism is perhaps the best known and most highly studied example of social parasitism. Obligate interspecific brood parasites are only able to reproduce by laying their eggs in the nests of other species, which then take care of the parasitic young (Davies 2000). Unique aspects of a brood parasitic lifestyle have long held the interest of biologists for evolutionary, ecological and ethological reasons. Firstly, the strong selection pressure acting on both parasite and host can potentially produce easily detectable adaptations in both members of the interaction making avian brood parasitism one of the best opportunities to study co-evolutionary interactions in vertebrates (Rothstein 1990). Secondly, brood parasites may exert potentially dramatic and detrimental effects on the reproductive success of many other species of birds (Ortega 1998). Thirdly, the interactions between avian brood parasites and their hosts are often characterized by a stunning diversity of sophisticated behavioural adaptations (Davies 2000).

Obligate brood parasitism is a rare phenomenon among birds and is found in only 1% of the approximately 10 000 avian species (Davies 2000). Yet, this reproductive strategy has evolved independently at least seven times (Jetz et al. 2012). The largest and most commonly known group are the cuckoos (Cuculidae), consisting of 52 species in two subfamilies that are mostly distributed throughout Europe and Asia. In Africa brood parasitism has evolved in two other families— the honeyguides (Indicatoridae) that have 20 parasitic species and two groups of finches (Viduidae) consisting of 17 species. In South America a single species of duck (*Heteronetta atricapilla*) has evolved an obligatory parasitic lifestyle. Lastly, throughout the

Americas are the cowbirds (Icteridae), the most notorious brood parasites, which consist of five parasitic species (Ortega 1998; Davies 2000).

As with conventional parasites, brood parasites may substantially lower the reproductive success of their hosts, however, their impact on their hosts or 'virulence' varies considerably depending on the strategies they employ to exploit their hosts (Kilner 2005). The most virulent are those that do not tolerate any host offspring (Soler and Soler 2017). For example, in species such as common cuckoos (*Cuculus canorus*) and honeyguides, the parasitic chick actively kills its foster siblings, thereby reducing the host's reproductive output to zero (Honza et al. 2007; Spottiswoode and Koorevaar 2011). This strategy is similar to other conventional parasites that castrate their hosts so that they can monopolize on the host's reproductive energy (e.g. Lafferty and Kuris 2009). Meanwhile, the brood parasitic black-headed duck is comparatively innocuous, with precocial young that leave their foster family soon after hatch and appear to impose almost no costs to their hosts (Odell and Eadie 2004). Other brood parasites, including the cowbirds, are raised alongside host nestmates and have been traditionally considered to be moderately virulent (Sorenson et al. 2003). However, their actual impact on their hosts may have been greatly underestimated. Cowbirds, in particular, may further reduce host fitness in a number of less obvious ways. Adult cowbird females will often remove a portion of the clutch in which they will lay (Sealy 1992). Parasitism itself may cause abandonment (Sedgwick and Knopf 1998; Harris 1991), or clutch size may be reduced past a critical threshold as to cause abandonment (Sealy 1992). Cowbird nestlings are also fierce competitors in the nest and their typically larger size and superior begging behaviour may allow them to outcompete host nestlings for parental food provisioning leading to lower quality fledglings (Lichtenstein and Sealy 1998; Dearborn 1998; Lorenzana and Sealy 1999) or even female-biased mortality in their

hosts (Zanette et al. 2005, 2012). Finally, adult cowbird females may destroy the contents of late-stage nests that they have not parasitized in order to return and parasitize the replacement nests at a more opportune time (Arcese et al. 1996).

In response to the threat of parasitism many host species have evolved some measure of defense. Several host species reject parasitic eggs from their nests (Ortega 1998) which may have led to the egg mimicry seen in parasitic species such as the common cuckoo (Brooke and Davies 1998). Other hosts may abandon an entire nest once it is parasitized or just bury the clutch (Davies 2000). Many other hosts readily accept parasitic eggs likely because the cost of rejection outweighs that of acceptance (summarized in Ortega 1998). Unlike parasitic eggs, the nestlings of cuckoos and cowbirds are almost universally accepted by their hosts (Rothstein 1990); however, recent evidence suggests that some host species discriminate against parasitic nestlings (Langmore et al. 2003; Schuetz 2005; Soler 2009; Sato et al. 2010; Tokue and Ueda 2010) or fledglings (Rasmussen and Sealy 2006). Some host species successfully prevent parasitism altogether by either refusing to leave the nest or aggressively attacking the parasitic intruder (Hobson and Sealy 1989), however, the effectiveness of this strategy in preventing parasitism is debatable (Ortega 1998; Swan personal observations).

Some avian brood parasites seem to adaptively induce behavioural changes in their hosts. One such strategy that imposes relatively little negative impact on host reproduction is the 'distraction' strategy of great spotted cuckoos (*Clamator glandarius*: Alvarez and Aras de Reyna 1974; Soler et al. 1999). Male cuckoos produce loud vocalizations while flying close to the nests of their magpie (*Pica pica*) hosts causing the incubating host females to leave the nest to chase after the cuckoo male. The cuckoo female, which has been waiting close by, then approaches the undefended nest and lays her own egg. Brood parasitic nestlings can also actively manipulate the

feeding behaviour of their host parents in a number of ways. Some parasitic nestlings may appropriately adjust their begging intensity to balance the begging competition within the nest (Rivers 2007) by mimicking the calls of either host nestlings (Langmore et al. 2008) or fledglings (De Marsico et al. 2012) or even mimicking the sound of an entire host brood (Davies et al. 1998; Gloag and Kacelnik 2013). One rather severe example of manipulative behaviour by brood parasites is that of the 'mafia' strategy. This involves the parasite, once finding that its egg has been rejected, retaliating by destroying the remaining host eggs/ nestlings (Zahavi 1979). By imposing extra fitness costs to rejecting parasitism, the parasite promotes future acceptance in their hosts. The mafia hypothesis has been experimentally corroborated in both the great spotted cuckoo (Soler et al. 1995, 1999) and brown-headed cowbirds (Hoover and Robinson 2007). A similar behavioural manipulation involves supposed farming behaviour by brood parasites (reviewed in detail in Chapters 2 and 3). The farming hypothesis suggests that brood parasites will destroy, or 'farm', host nests found too late in the nesting cycle for it to be suitable for parasitism, thereby inducing those hosts to begin a new nesting attempt that the parasite can return to lay in at a more appropriate time (Arcese et al. 1992; 1996).

1.6 Animal home ranges and home range estimators

Key questions in ecology involve how the spatial distribution of individuals are influenced by interactions with others and their environment (Lima and Zollner 1996; Turchin 1998; Wiegand et al. 1999; Matthiopoulos 2003). Data on the space use of wildlife are ordinarily represented by a series of location points obtained over time through some tracking technology such as radio-telemetry or some global positioning system (GPS) device. These locations can be used to map spatial boundaries and intensity of space use which can then be analyzed (White and

Garrott 1990). In brood parasitic systems, in particular, home range analyses may be especially informative on how parasites find, select and defend the nests of their hosts. These are the central topics I address in Chapter 4.

A home range is customarily defined as the more or less confined area where an animal enacts its regular activities of food gathering, mating, rearing young, etc. and requires some degree of site fidelity (Burt 1943; Powell 2000). Stamps (1995) argued that animals occupy home ranges so that individuals can better learn the locations of critical resources or escape routes. This requires that animals form some type of spatial map of where they live that includes where different resources and other features are located within their home range. This cognitive spatial map must change over time as an animal learns new details about its environment (Powell 2000). A territory may be defined as an area within a home range to which an animal has more or less exclusive access (Powell 2000). This usually requires active defense in some way or another of at least a portion of an animal's home range. Almost always, animals are territorial only when they have a critical and limiting resource.

Historically, the most common used method of estimating home range boundaries has been the minimum convex polygon (MCP: Mohr 1947). Although some other techniques have been proposed, MCPs are usually constructed by connecting the outermost points in a sample of location points. Given the simplicity of design and lack of variation in construction, MCPs may be the only home range estimates that can be reliably compared among studies (Harris et al. 1990). Even so, MCPs have three main disadvantages: 1) they provide almost no insight into the relative use of space within a home range; 2) the may contain large areas that were never visited by the subject and; 3) they are greatly influenced by sample size along with peripheral and outlining locations (Harris et al. 1990).

Modern computational power now allows researchers to create much more appropriate home range models such as the utilization distribution (van Winkle 1975). A utilization distribution (UD) is a probability density function (Silverman 1986) that quantifies the relative use of space for an individual based upon a sample of location points (Kernohan et al. 2001). Not only do UDs approximate the size and boundaries of home ranges, but also depict the probability of an individual occurring at any given location within their home range (White and Garrott 1990). UDs are ideal models for analyzing the space use of animals because individuals rarely use all parts of their home range uniformly. Rather, some areas are commonly occupied (e.g. nest site, location of food) while others are rarely visited (Marzluff et al. 1997). UDs can be plotted as a series of isopleths that represent areas of equal use in a similar way topographic maps use isopleths to depict equal elevation. Home range boundaries, for example, are typically represented as the 95th density isopleth for an individual representing a 95% probability of finding that individual within that boundary (Powell 2000). Using a distribution of relative use instead of discrete location data in space use reduces error from telemetry accuracy and allows for predictions in locations where animals were never actually observed because of discontinuous monitoring (Kernohan et al. 2001; Moser and Garton 2007). Studies may use UDs to link probability of use with some habitat variable(s) given that the height of a UD represents the relative probability of an individual being at any given location (Marzluff et al. 2004; Millspaugh et al 2006). Others estimate spatial overlap among individuals by overlaying multiple distributions (e.g. Simpfendorfer et al. 2012; Cooper et al. 2014).

A reoccurring source of contention with any space-use study, whether they use UD or MCP estimators, is with the sampling protocol, specifically whether the location points using in the analysis are statistically independent. Because of logistical restrictions in sampling method

and the biology of the species being followed, achieving statistical independence can be difficult (Smith et al. 1981). It has traditionally been accepted that statistical independence of location points is generally achieved when the data is not serially autocorrelated (i.e. the location at a given time is not dependent on the previous location: Schoener 1981). When estimating home range size, for example, some studies suggest that autocorrelated locations may result in underestimates (Schoener 1981; Swihart and Slade 1985), however, others have not found this to be the case (de Solla et al. 1999). Depending on the species being monitored this restriction on sampling may be unreasonably limiting, allowing for few location points to be recorded over time. If the purpose of a study is to estimate the internal configuration of space use within an animal's range (Marzluff et al. 2004), it seems much more reasonable to be concerned with biological rather than statistical independence of points (Barg et al. 2005). Biological independence is defined as any sampling interval long enough to allow an animal to travel from one point in its range to another (Lair 1987; Solla et al. 1999). This condition on sampling protocol may better accommodate species-specific movement abilities (Andersen and Rongstad 1989). For these reasons, in chapter 4 I specifically disregard the need for statistical independence in my location sampling, instead using a 'burst' sampling method (Barg et al. 2005) that maximized the number of points I was able to record.

Obligate avian brood parasitizes that lay their eggs in the nests other species offer a unique model species for studying space use because, unlike other birds, they are not obliged to return to a set nesting site. Instead, freedom from parental care allows them to separate activities, such as mating, nest searching and feeding, both temporally and spatially (Thompson 1994). Nest searching is performed almost exclusively by females and there is strong selection pressure for them to remember both the location and developmental stage of host nests (Davies 2000).

This is reflected in the enlarged hippocampus of female cowbirds relative to males (Sherry et al. 1992; Reboreda et al. 1996) and female cowbirds' ability to perform complex memory tasks (Guigueno et al. 2014). In other words, the cognitive spatial map of their home ranges must be continually updated for female brood parasites because suitable host nests are repeatedly emerging and disappearing. These topics are addressed in detail in chapter 4.

1.7 Study species: Brown-headed Cowbird (*Molothrus ater*)

The brown-headed cowbird is the most studied brood parasite in the world. Cowbirds are highly abundant throughout North America and are considered to be extreme host generalists, successfully parasitizing over 170 species of passerines (Rothstein 1994; Ortega 1998; Lower 2012). Brown-headed cowbirds can be found primarily in disturbed habitats that have high concentrations of food (Ortega 1998; Davies 2000). Interest in cowbirds has been spurred by the population declines of several migratory songbird species and the perceived role that cowbirds play in those declines (e.g. Smith et al. 2002; 2003). These concerns arouse primarily because of their overall abundance, high laying potential, competitive ability of their relatively large nestlings, and tendency to remove eggs while parasitizing (summarized in Ortega 1998). There is also an ever increasing amount of anecdotal, observational and experimental evidence suggesting that brown-headed cowbirds act as predators on non-parasitized host nests possibly to improve their reproductive success (Reviewed in Chapter 2 and 3). An individual brown-headed cowbird may be capable of laying up to 80 eggs in one season (Scott and Ankley 1983), however, the actual number laid may be significantly less (Alderson et al. 1999) suggesting that the number of host nests may represent a limiting reproductive resource. Despite this premium on available nests, female territoriality and how they find nests remains poorly understood (see Chapter 4).

Generally, as with all parasites, brown-headed cowbirds are entirely dependent on encountering hosts that are suitable for parasitism at the appropriate time.

1.8 References

Adamo SA. 2012. The strings of the puppet master: how parasites change host behaviour. In: Hughes D and Thomas F, editors. Parasitic Manipulation. Oxford: Oxford University Press.

Alderson GW, Gibbs HL and Sealy SG. 1999. Determining the reproductive behaviour of individual brown-headed cowbirds using microsatellite DNA markers. Anim. Behav. 58:895-905.

Alloway 1979 Alloway, T.M. 1979. Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). Anim. Behav. 27:202-210.

Alvarez F and Aras de Reyna L. 1974. Mecanismos de parasitiacion por *Clamator glandarius* y defense por *Pica pica*. Donana. Acta. Vertebrata. 1:43-65.

Andersen DE and Rongstad OJ. 1989. Home-range estimates of red-tailed hawks based on random and systematic relocations. J. Wildlife. Manage. 53:802-807.

Arcese P, Smith JMN, Hochachka WM, Rogers CM, Ludwig D. 1992. Stability, regulation, and the determination of abundance in an insular song sparrow population. Ecology. 73:805-822.

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. Proc Natl Acad Sci. U.S.A. 93:4608-4611.

Barg JJ, Jones J and Robertson RJ. 2005. Describing breeding territories of migratory passerines: Suggestions for sampling, choice of estimator, and delineation of core areas. J. Anim. Ecol. 74:139-149.

Beckage NE. 1997. Parasites and Pathogens: Effects on Host Hormones and Behavior. New York, NY: Chapman and Hall.

Berdoy M, Webster JP, Macdonald DW. 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. Proc. R. Soc. Lond. B. 267:1591-1594.

Brooke M and Davies NB. 1998. Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. Nature. 335:630-632.

Brooke M, Davies NB. and Noble DG. 1998. Rapid decline of host defenses in response to reduced cuckoo parasitism: behavioural flexibility of reed warblers in a changing world. Proc. R. Soc. 265:1277-1282.

Brooker M and Brooker L. 1996. Acceptance by the splendid fairy-wren of parasitism by Horsfield's bronze-cuckoo: further evidence for evolutionary equilibrium in brood parasitism. Behav. Ecol. 7:395-407.

Broughton KE, Middleton ALA, and Bailey ED. 1987. Early vocalizations of the Brown-headed Cowbirds and three host species. Bird. Behav. 7:27-30.

Burt WH. 1943. Territoriality and home range concepts as applied to mammals. J. Mammol. 24:346-352.

Buschinger A. 1976. Eine Methode zur Zucht der Gastameise *Formicoxenus nitidulus* (Nyl.) mit *Leptothorax acervorum* (Fabr.) als "Wirtsameise" (Hym., Form.). Insectes. Soc. 23:205-214.

Buschinger A. 1986. Evolution of social parasitism in ants. Trends. Ecol. Evol. 1: 155-160.

Buschinger A, Jessen K and Cagniant, H. 1990. The life history of *Epimyrma algeriana*, a slave-making ant with facultative polygyny (Hymenoptera: Formicidae). Zool. Beitr. 33:23-49.

Carney WP. 1969. Behavioral and morphological changes in carpenter ants harboring *Dicrocoelid metacercariae.* Amer. Midland Naturalist. 82:605-611.

Cator LJ, Lynch PA, Read AF and Thomas MB. 2012 Do malaria parasites manipulate mosquitoes? Trends Parasitol. 28:466-470.

Cator LJ, Lynch PA, Thomas MB and Read AF. 2014. Alterations in mosquito behaviour by malaria parasites: potential impact on force of infection. Malar. J. 13:164.

Cervo R, Macinai V, Dechigi F and Turillazzi S. 2004. Fast growth of immature brood in a social parasite wasp: a convergent evolution between avian and insect cuckoos. Am. Nat. 164:814-820.

Cooper NW, Sherry TW, and Marra PP. 2014. Modeling three-dimensional space use and overlap in birds. Auk. 131:681-693.

D'Ettorre P, Heinze J, Schulz C, Fzrancke W and Ayasse M. 2004. Does she smell like a queen? Chemoreception of a cuticular hydrocarbon signal in the ant *Pachycondyla inversa*. J. Exp. Biol. 207:1085-1091.

Dantzer R. 2001. Cytokine-induced sickness behavior: where do we stand? Brain Behav. Immun. 15:7-24.

Darwin C. 1859. On the origin of species. Harvard University Press, Cambridge Mass.

Davies NB. 2000. Cuckoos, cowbirds and other cheats. London, UK: Oxford University Press.

Davies SK and Sealy SG. 1998. Nesting biology of the Baird's Sparrow in southwestern Manitoba. Wilson Bull. 110:262-270.

Dawkins R. 1990. Parasites, desiderata lists and the paradox of the organism. Parasitolog.y 100:63-73.

Dawkins R. 1982. The extended phenotype. Oxford, UK: Oxford University Press.

Dawkins, R., and Krebs, J.R. 1979. Arms race between and within species. Proc. R. Soc. 205: 489-511.

De Marsico MC, Gantchoff MG and Reboreda JC. 2012. Host parasite coevolution beyond the nestling stage? Mimicry of host fledglings by the specialist screaming cowbird. Proc. R. Soc. B. 279:3401-3408.

de Solla SR, Bonduriansky R and Brooks RJ. 1999. Eliminating autocorrelation reduces biological relevance of home range estimates. J. Anim. Ecol. 68:221-234.

Dearborn DC. 1998. Begging behavior and food acquisition by brown-headed cowbird nestlings. Behav. Ecol. Sociobiol. 43:259-270.

Esch W and Fernandez JC. 1993. A functional biology of parasitism: ecological and evolutionary implications. 337pp.

Fischer B and Foitzik S. 2004. Local co-adaptation leading to a geographical mosaic of coevolution in a social parasite system. J. Evol. Biol. 17:1026-1034.

Fischer-Blass B, Heinze J and Foitzik S. 2006. Microsatellite analysis reveals strong but differential impact of a social parasite on its two host species. Mol. Ecol. 15: 863-872.

Flegr J. 2007. Effects of toxoplasma on human behavior. Schizophr Bull. 33:757-760.

Foitzik S and Herbers JM. 2001. Colony structure of a slavemaking ant. I. Intracolony relatedness, worker reproduction, and polydomy. Evolution 55:307-315.

Foitzik S, Fischer B and Heinze J. 2003. Arms race between social parasites and their hosts: geographic patterns of manipulation and resistance. Behav. Ecol. 14:80-88.

Gibbs HL, Sorenson MD, Marchetti K, Brooke MD, Davies NB, and Nakamura H. 2000. Genetic evidence for female host-specific races of the common cuckoo. Nature 407:183-186.

Gladstone DE. 1981. Why there are no slave rebellions. Am. Nat. 117:779-781.

Gloag R and Kacelnik A. 2013. Host manipulation via begging call structure in the brood-parasitic shiny cowbird. Anim. Behav. 86:101-109.

Gould SJ and Lewontin RC. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist program. Proc. R. Soc. Lond. 205:581-598.

Guigueno MF, Snow DA, MacDuogall-Shackleton SA and Sherry D. 2014. Female cowbirds have more accurate spatial memories than males. Biol. Letters.

Hamilton WD. 1964. The genetical evolution of social behaviour: I and II. J. Theor. Biol. 7:1-52.

Hare JF and Alloway TM. 2001. Prudent *Protomognathus* and despotic *Leptothorax duloticus*: differential costs of ant slavery. Proc. Natl. Acad. Sci. USA. 98:12093-12096.

Harris JH. 1991. Effects of brood parasitism by brown-headed cowbirds on willow flycatcher nesting success along the Kern river, California. Western Birds 22:13-26.

Harris S, Cresswell WJ, Forde PG, Trewhella WJ, Woollard T and Wray S. 1990. Home-range analysis using radio-tracking data – a review of problems and techniques particularly as applied to the study of mammals. Mammal. Review. 20:97-123.

Heil M. 2016. Host manipulation by parasites: causes, patterns, and remaining doubts. Front. Ecol. Evol. 80:1-15.

Hughes DP, Brodeur J and Thomas F. 2012. Host manipulation by parasites. Oxford: Oxford University Press.

Hobson KA and Sealy SG. 1989. Cowbird egg buried by a northern oriole. J. Field Ornithol. 58:222-224.

Hölldobler B and Wilson EO. 1990. The ants. Harvard University Press, Cambridge, Massachusetts, U.S.A.

Holmes JC and Bethel WM. 1972. Modification of intermediate host behavior by parasites. In: Canning EU and Wright CA, editors. Behavioral Aspects of Parasite Transmission. London, UK: Academic Press, London. Pp.123-149.

Honza M, Poláiková L and Procházka P. 2007. Ultraviolet and green parts of the colour spectrum affect egg rejection in the song thrush (*Turdus philomelos*). Biol. J. Linnean Soc. 92:269-276.

Hoover JP and Robinson SK. 2007. Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. Proc. Nat. Acad. Sci. U.S.A. 104:4479‑4483.

Howard RW, McDaniel CA and Blomquist GJ. 1980. Chemical mimicry as an integrating mechanism: cuticular hydrocarbons of a termitophile and its host. Science 210:431-433.

Howard RW, Pérez-Lachaud G and Lachaud JP. 2001. Cuticular hydrocarbons of *Kapala sulcifacies* (Hymenoptera: Eucharitidae) and its host, the ponerine ant *Ectatomma ruidum* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 94: 707-716.

Hrdy SB. 1974. Male-male competition and infanticide among the langurs (*Presbytis entellus*) of Abu, Rajasthan. Folia. Primatol. 22: 19-58.

Hrdy SB. 1977. The langurs of Abu: Female and male strategies of reproduction. Harvard University Press, Cambridge, MA.

James WH. 2010. Potential solutions to problems posed by the offspring ratios of people with parasitic and viral infections. Folia Parasitol. 57:114-120.

Jetz W, Thomas G, Hartmann A and Mooers O. 2012. The global diversity of birds in space and time. Nature 491:444-448.

Kernohan BJ, Gitzen RA and Millspaugh JJ. 2001. Analysis of animal space use and movements. In Millspaugh JJ and Marzluff JM, editors. Radio tracking and animal populations. Academic, San Diego, California, USA. Pp 125-166.

Kilner RM. 2005. The evolution of virulence in brood parasites. Ornithol. Sci. 4:55-64.

Klein SL. 2003. Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. Physiol. Behav 79:441-449.

Klein SL. 2005. Parasite manipulation of host behaviour: mechanisms, ecology, and future directions. Behav. Processes. 68:219-221.

Lack D. 1968. Ecological adaptations for breeding in birds. London: Methuen and Co. Ltd.

Lafferty KD. 1999. The evolution of trophic parasitism. Parasitol. Today. 15:111-115.

Lafferty KD. 2006. Can the common brain parasite, *Toxoplasma gondii*, influence human culture? Proc. Roy. Soc. 273:2749-2755.

Lafferty KD and Kuris AM. 2009. Parasitic castration: the evolution and ecology of body snatchers. Trends in Parasitol. 25:564-572.

Lair H. 1987. Estimating the location of the focal center in red squirrel home ranges. Ecology 68:1092-1101.

Langmore NE, Maurer G, Adcock GL and Kilner RM. 2008. Socially acquired host-specific mimicry and the evolution of host races in Horsfield's bronze- cuckoo *Chalcites basalis*. Evolution 62:1689-1699.

Langmore NE, Hunt S and Kilner RM. 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. Nature 422:157-160.

Lefevre T, Roche B, Poulin R, Hurd H, Renaud F and Thomas F. 2008. Exploiting host compensatory responses: The 'must' of manipulation? Trends Parasitol. 24:435-439.

Lichtenstein G and Sealy SG. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. Proc. Roy. Soc. B. 265:249-254.

Lima SL and Zollner PA. 1996. Towards a behavioural ecology of ecological landscapes. Trends Ecol. Evol. 11:131-135.

Lorenzana J and Sealy SG. 1999. A meta-analysis of the impact of parasitism by the brown-headed cowbird on its hosts. Studies Avian Biol. 18:241-253.

Lowther PE. 1993. Brown-headed cowbirds (*Molothrus ater*). In A Poole and F Gill eds, The Birds of North America no 47. Philadelphia PA, American Ornithologists Union, Washington DC.

Marzluff JM, Millspaugh JJ, Hurvitz P and Handcock MS. 2004. Resource utilization of an avian nest predator: relating resources to a probabilistic measure of animal use. Ecology. 85:1411-1427.

Marzluff JM, Millspaugh JJ, Hurvitz P and Handcock MS. 2004. Relating resources to a probabilistic measure of space use: forest fragments and Steller's jays. Ecology. 85:1411-1427.

Matthiopoulos, J. 2003. The use of space by animals as a function of accessibility and preference. Ecol. Modelling. 159:239-268.

Maynard Smith, J. 1982. Evolution and theory of games. Cambridge University Press, New York. 224 pp.

Millspaugh JJ, Nielson RM, McDonald L, Marzluff JM, Gitzen RA, Rittenhouse CD, Hubbard MW and Sheriff SL. 2006. Analysis of resource selection using utilization distributions. J. Wildlife Manage. 70:384-395.

Mohr CO. 1947. Table of equivalent populations of North American small mammals. Amer. Mid. Naturalist 37:223-249.

Moksnes A and Røskaft E. 1995. Egg-morphs and host preference in the common cuckoo (*Cuculus canorus*): an analysis of cuckoo and host eggs from European museum collections. J. Zool. 236:625-648.

Moore J. 2002. Parasites and the behavior of animals. Oxford, UK: Oxford University Press.

Mori A, Grasso DA and Le Moli F. 2000. Raiding and foraging behavior of the blood-red ant, *Formica sanguinea* Latr. (Hymenoptera, Formicidae). J. Insect Behav. 13:421-438.

Moser BW and Garton EO. 2007. Effects of telemetry location error on space-use estimate using a fixed-kernel density estimator. J. Wildlife Manage. 71:2421-2426.

Nickol BB. 2005. Parasitic manipulation: should we go anywhere? Behav Process. 68:201-203.

Odell NS and Eadie JM. 2010. Do wood ducks use the quantity of eggs in a nest as a cue to the nest's value. Behav Ecol. 21:794-801.

Ortega CP. 1998. Cowbirds and other brood parasites. The University of Arizona Press, Tucson, Arizona, U.S.A.

Packer C and Pusey AE. 1983. Male takeovers and female reproductive parameters: a simulation of oestrous synchrony in lions (*Panthera leo*). Anim. Behav. 31:334-340.

Palombit RA. 2015. Infanticide as sexual conflict: Coevolution of males strategies and female counterstrategies. Cold Spring Harbour Perspective Biology 7:a017640.

Petrie M and Møller AP. 1991. Laying eggs in others' nests: intraspecific brood parasitism in birds. Trends Ecol. Evol. 6:315-319.

Poulin R 1994. Meta-analysis of parasite-induced behavioural changes. Anim. Behav. 48:137-146.

Poulin R. 1999. The functional importance of parasites in animal communities: many roles at many levels. International J. Parasitol. 29:903-914.

Poulin R. 2000. Manipulation of host behaviour by parasites: a weakening paradigm? Proc R Soc B. 267:787-792.

Poulin R. 2007. Evolutionary ecology of parasites. Princeton, USA: Princeton University Press.

Pusey and Packer 1994. Infanticide in lions: consequences and counterstrategies. In Infanticide and parental care.

Rasmussen JL and Sealy SG. 2006. Hosts feeding only brown-headed cowbird fledglings: where are the host fledglings? J. Field Ornithol. 77:269-279.

Reboreda JC, Clayton N and Kacelnik A. 1996. Species and sex differences in hippocampus size in parasitic and non-parasitic cowbirds. Neuroreport 7:505-508.

Reiber C, Shattuck EC, Fiore S, Alperin P, Davies V and Morre J. 2010. Change in human social behaviour in response to a common vaccine. 20:729-733.

Rivers JW. 2007. Nest mate size, but not short-term need, influences begging behavior of a generalist brood parasite. Behav. Ecol. 18:222-230.

Rogers ME and Bates PA. 2007. *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. PLoS Pathog. 3:818-825.

Rothstein SI. 1990. A model system for coevolution: avian brood parasitism. Ann. Rev. Ecol. Syst. 21:481-508.

Rozsa L. 1992. Points in question endangered species. International J. Parasitol. 22:265-266.

Sato T. 1986. A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika. Nature 323:58-59.

Sato NJ, Tokue K, Noske RA, Mikami OK and Ueda K. 2010. Evicting cuckoo nestlings from the nest: a new anti-parasitism behaviour. Biology Letters 6:67-69.

Schaller, G. B. 1972. The Serengeti lion. Chicago: University of Chicago Press.

Schuetz JG. 2005. Reduced growth but not survival of chicks with altered gape patterns: implications for the evolution of nestling similarity in a parasitic finch. Anim. Behav. 70:839-848.

Scott DM and Ankney CD. 1983. The laying cycle of Brown-headed Cowbirds. Condor. 94:579-584.

Sealy SG. 1992. Removal of yellow warbler eggs in association with cowbird parasitism. Condor. 94:40-54.

Sedgwick JA and Knopf FL. 1998. A high incidence of brown-headed cowbird parasitism of willow flycatchers. Condor. 90:253-256.

Sherman PW. 1981. Reproductive competition and infanticide in Belding's ground squirrels and other animals. In Alexander RD and Tinkle DW, editors. Natural selection and social behavior. Chiron, New York. pp. 311-331.

Sherry D., Forbes M., Khurgel M. and Ivy G. 1992. Females have a larger hippocampus than males in the brood-parasitic Brown-headed Cowbird. Proc. Nat. Acad. Sci. USA 90:7839-7843.

Silverman BW. 1986. Density estimation for statistics and data analysis. Chapman and Hall, New York, New York, USA.

Simpfendorfer CA, Olsen EM, Heupel MR and Moland E. 2012. Three-dimensional kernel utilization distributions improve estimates of space use in aquatic animals. Can J Fish Aquatic Sci 69:565-572.

Smith GJ, Cary JR and Rongstad OJ. 1981. Sampling strategies for radio-tracking coyotes. Wildlife Soc. Bull. 9:88-93.

Smith JNM, Taitt MJ, Zanette L and Myers‐Smith IH. 2003. How do brown‐headed cowbirds (*Molothrus ater*) cause nest failures in song sparrows (*Melospiza melodia*)? A removal experiment. Auk. 120:772‐783.

Smith JNM, Taitt MJ and Zanette L. 2002. Removing brown-headed cowbirds increases seasonal fecundity and population growth in song sparrows. Ecology. 83:3037-3047.

Soler JJ and Soler M. 2017. Evolutionary change: facultative virulence by brood parasites and tolerance and plastic resistance by hosts. Anim. Behav. 125:101-107.

Soler JJ, Sorci G, Soler M and Møller AP. 1999. Change in host rejection behavior mediated by the predatory behavior of its brood parasite. Behav. Ecol. 10:275-280.

Soler M, Soler JJ, Martinez JG, Møller AP. 1995. Magpie host manipulation by great spotted cuckoos: evidence for an avian mafia? Evolution. 49:770-775.

Soler M. 2009. Co-evolutionary arms race between brood parasites and their hosts at the nestling stage. J. Avian Biol. 40:237-240.

Sorenson MD, Sefc KM and Payne RB. 2003. Speciation by host switch in brood parasitic indigobirds. Nature 424:928-931.

Spottiswoode CN and Koorevaar J. 2011. A stab in the dark: chick killing by brood parasitic honeyguides. Biology Letters 10.1098/rsbl.2011.0739.

Stoddard MC and Stevens M. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. Proc R Soc Lond B. 277:1387-1393.

Stoddard MC, Stevens M. 2011. Avian vision and the evolution of egg color mimicry in the common cuckoo. Evolution. 65:2004-2013.

Stuart RJ and Alloway TM. 1985. Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). Anim. Behav. 33:1080-1088.

Swihart RK and Slade NA. 1985. Influence of sampling interval on estimates of home-range size. J. Wildlife Manage. 49:1019-1025.

Tallamy DW. 1985. Egg-dumping in lace bugs (Hemiptera: Tingidae). Behav. Ecol. Sociobiol. 17:357-362.

Thomas F, Adamo S, Moore J. 2005. Parasitic manipulation: where we are and where we should go? Behav Process. 68:185-199.

Thompson FR III. 1994. Temporal and spatial patterns of breeding Brown-headed Cowbirds in the midwestern United States. Auk 111:979-990.

Thompson FR, Dijal W and Burhans DE 1999. Video identification of predators at songbird nests in old fields. Auk 116:259-264.

Toft CA. 1986. Communities of parasites with parasitic life-styles. In Diamond JM and Case TJ, editors. Community Ecology. Harper and Row. pp. 445-463.

Tokue K and Ueda K. 2010. Mangrove geryones *Gerygone laevigaster* eject little bronze-cuckoo *Chalcites minutillus* hatchlings from parasitized nests. Ibis 152:835-839.

Topoff H and Zimmerli E. 1993. Colony takeover by a socially parasitic ant, *Polyergus breviceps*: the chemicals obtained during host-queen killing. Anim. Behav. 46:479-486

Trivers RL. 1971. The evolution of reciprocal altruism. Q. Rev. Biol. 46:35-57.

Turchin P. 1998. Quantitative Analysis of Movement. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Van Valen L. 1973. A new evolutionary law. Evolutionary Theory 1:1-30.

Van Winkle W. 1975. Comparison of several probabilistic home-range models. J. Wildlife Manage. 39:118–123.

White GC and Garrott RA. 1990. Analysis of wildlife radio-tracking data. Academic Press, San Diego California, USA.

Wickler W. 1976. Evolution-oriented ethology, kin selection, and altruistic parasites. Zeitschrift fur Tierpsychologie. 42:206-214.

Wiegand T, Moloney KA, Naves J and Knauer, F. 1999. Finding the missing link between landscape structure and population dynamics: a spatially explicit perspective. Am. Nat. 154:605-627.

Wilson, E.O. 1971. The insect societies. The Belknap Press of Harvard University Press, Cambridge, U.S.A.

Wilson EO. 1975. *Leptothorax duloticus* and the beginnings of slavery in ants. Evolution 29: 108-119.

Wisenden BD. 1999. Alloparental care in fishes. Reviews in Fish Biology and Fisheries 9:45-70.

Wolff JO and MacDonald DW. 2004. Promiscuous females protect their offspring. Trends Ecol. Evol. 19:127-134.

Wood CL, Byers JE, Cottingham KL, Altman I, Donahue MJ and Blakeslee AMH. 2007. Parasites alter community structure. Proc. Nat. Acad. Sci. 104: 9335-9339.

Worth AR, Lymbery AJ and Thompson RCA. 2013. Adaptive host manipulation by *Toxoplasma gondii*: fact or fiction? Trends Parasitol. 29:150-155.

Zahavi A. 1979. Parasitism and nest predation in parasitic cuckoos. Am. Nat. 113:157-159.

Zanette L, MacDougall-Shackleton E, Clinchy M and Smith JNM. 2005. Brown-headed cowbirds skew host offspring sex ratios. Ecology 86:815-820.

Zanette LY, Clinchy M, Leonard ML, Horn AG, Haydon DT and Hampson E. 2012. Brood-parasite-induced female-biased mortality affects songbird demography: negative implications for conservation. Oikos 121:1493-1500.

Chapter 2

2 Interspecific infanticide by the brown-headed cowbird: an evaluation of the farming hypothesis

2.1 Introduction

Systematic infanticide, despite offending our sensibilities, may prove an important and common strategy across a wide range of taxa including primates (Hrdy 1977; Hitaiwa-Hasegawa and Hiraiwa 1994), rodents (Sherman 1981; Elwood and Kennedy 1994; Hoogland 2007), carnivores (Packer and Pusey 1984), cetaceans (Patterson et al. 1998) and birds (Veiga 2000). Several hypotheses, none of which are mutually exclusive, try to explain how infanticide may prove beneficial, such as providing nutritional benefits (predation hypothesis: Palombit 2015) or improving access to resources for the infanticidal individual or their offspring (resource competition hypothesis: Agoramoorthy and Rudran 1995). Hrdy (1974) first suggested that killing of unrelated conspecific young by adult males may serve to increase their reproductive success (sexual selection hypothesis). Essentially, hostile males coerce females to become sexually receptive early by killing their dependent offspring. Although it has become widely accepted that infanticide may yield a reproductive advantage in this way, rigorous evidence in support of this hypothesis has only been obtained in a few species (Pusey and Packer 1994). An excellent and well-studied example of sexually selected infanticide may be seen in male lions (*Panthera leo*) that seize control of a new pride, kill but do not consume all small cubs, and then

mate with the newly sexually receptive females (Schaller 1972; Bertram 1975; Packer and Pusey 1984; 1987; Packer et al. 1988). In a striking parallel, some brood parasitic birds may also kill the eggs and chicks of their hosts with similar reproductive outcomes.

Obligate avian brood parasites, such as cuckoos and cowbirds, lay their eggs in the nests of other species, after which the 'hosts' provide all parental care for the parasitic young (Davies 2000). Female brood parasites must synchronize their laying with that of their hosts in order to ensure adequate incubation for their egg to hatch (Fiorini et al. 2009). Late-stage nests are effectively unavailable to a brood parasite much like females out of estrous are unavailable to invading male lions. Also, just as a lion creates mating opportunities through infanticide, a brood parasite may kill eggs or nestlings when they encounter a nest too far into incubation for successful parasitism (Granfors et al. 2001; Peer 2006; Dubina and Peer 2013; Swan et al. 2015)— forcing the host to renest earlier than it otherwise would and providing the perpetrator with another opportunity for parasitism (Arcese et al. 1996). This 'farming' hypothesis is theoretically enticing, however, there remain significant gaps in the evidence needed to conclude that killing host young represents a reproductive adaptation analogous to that of infanticide observed in other species.

Here, I examine infanticidal behaviour by a brood parasite in order to provide a comprehensive evaluation of the fundamental requirements of the farming hypothesis. Based on infanticide literature I assert that, at a minimum, three critical conditions must be satisfied if we are to accept that attacking host nests represents an adaptive reproductive strategy. First and foremost, it is necessary to establish that infanticide by brood parasites is not uncommon, but an ordinary aspect of a parasitic lifestyle. Several lines of evidence suggest that brood parasites destroy but do not consume the contents of nests (eggs and nestlings) that are not parasitized,

however, most of the available data are either circumstantial or entails anecdotal reports of a single event (Tate 1967, Dow 1972, Arcese et al. 1992; 1996, Scott et al. 1992, Sealy 1992, 1994, Dearborn 1996, Elliot 1999, Thompson et al. 1999, Granfors et al. 2001, Smith et al. 2002, Zanette et al. 2003, 2011, Briskie 2007, Hoover and Robinson 2007, Thompson 2007, Dubina and Peer 2013). Likely because of logistical constraints in identifying the species of nest predator, few direct data are available on the frequency of these attacks. Early reports of infanticide by male lions were also rarely observed directly because of their nocturnal tendencies (Pusey and Packer 1994), but have since been estimated to be among the most common causes of cub mortality (Bertram 1975; Packer and Pusey 1983, 1984; Packer et al. 1988). The second condition is that attacks by a brood parasite should not be indiscriminate, but focused on late-stage nests that have not previously been parasitized by that individual (Arcese et al. 1996). This is similar to male lions killing only cubs that are not their own (Packer and Pusey 1984; Packer and Pusey 1994). Evidence showing that brood parasites selectively attack non-parasitized nests, however, is currently inconsistent. Higher predation rates generally of parasitized than non-parasitized nests have occasionally been reported (Arcese et al. 1996, Clotfelter and Yasukawa 1999, Hauber 2000), however, this trend is by no means universal (Ortega 1998, Table 7.6). My third condition requires that the brood parasite derives actual reproductive benefit from their infanticidal action. Just as sexually selected infanticide hinges upon male lions mating with the mothers of the cubs they kill (Gilbert et al. 1991), the farming hypothesis predicts high rates of parasitism in the replacement nests of the hosts that lost their nests to an infanticidal brood parasite (Hoover and Robinson 2007).

Other than farming, multiple hypotheses have been proposed to explain egg puncture/ removal by brood parasites, however, most apply to only specific circumstances and rarely

involve the total destruction of a clutch or brood (reviewed in Peer 2006). Similar to infanticidal lions that rarely consume their targets (Packer and Pusey 1994), nutrition seems an unlikely motivator for nest attacks by brood parasites (Sulc et al. 2016). Some brood parasites may employ mafia-like tactics and cause nests to fail if their own egg is rejected (Hoover and Robinson 2007), however, this scenario only applies to particular incidents involving host species that eject parasitic eggs. Partial removal of a clutch may enhance the incubation efficiency for a parasitic egg (Peer and Bollinger 2000) or reduce competition for a parasitic nestling (Llambias et al. 2006). These circumstances require that the nest is parasitized (i.e. the parasite lays an egg in it) shortly before, after or during the egg puncture/ removal event, and should ideally not fail as a result. Occasionally a partial reduction in clutch size or the addition of a cowbird egg may cause hosts to abandon their nests (Peer 2006) and I consider these nest failures as failed attempts at parasitism as opposed to farming events. In contrast to egg puncture/ removal events in early-stage host nests associated with egg-laying by the parasite, that may accidentally cause nest failure, the total destruction of all eggs or nestlings in late-stage nests (that are unsuitable to lay in) is much more clearly suggestive of infanticide being the purpose, rather than the by-product, of such an act. Experimental evidence corroborates that female brood parasites discriminate among nests depending on their developmental age and cause more damage to late-stage clutches, consistent with the principal purpose of damaging late-stage clutches being to commit infanticide to induce re-nesting (Swan et al. 2015).

Although it is possible to quantify the reproductive output of a brood parasite in the field, it is not necessarily feasible to compare the fitness of an individual that follows a farming strategy to one that does not. Moreover, the degree of behavioural sophistication necessary to make a farming strategy worthwhile cannot be readily assessed from field data alone. In

particular, do farmers benefit simply by increasing the number of nests available for parasitism or do they also improve their chance of discovering the specific replacement nests they create through their predatory actions? Apparent advanced spatial processing ability in female brood parasites (Sherry et al. 1992; Reboreda et al. 1996; Guigueno et al. 2014) may allow them to approximate the location and timing of a re-nesting attempt they create. Analytical and simulation models have previously proven useful when comparing hypothetical behavioural strategies among brood parasites and their hosts (e.g. Zanette et al. 2007; Gloag et al 2011; Chakra et al. 2014; Hauber 2014) and may be applied in a similar fashion to estimate the relative benefits of different farming strategies. Here, I utilize a Monte Carlo simulation model to compare the reproductive output of the following three hypothetical types of brood parasite: 1) the "non-farmer" that does not attack host nests; 2) the "simple farmer" that attacks late-stage non-parasitized host nests, increasing the number of laying opportunities available, but with no subsequent change in nest discovery and; 3) the "advanced farmer" that attacks host nests and reliably returns to parasitize the re-nesting opportunity they create thereby increasing their chance of nest discovery. Comparing the fitness payoffs among these strategies under different conditions allows one to consider whether 'farming' one's hosts is worthwhile, the necessary complexity of the behaviour, and how ecological variables may influence reproductive output.

This study aims to provide a comprehensive evaluation of the basic requirements of the farming hypothesis using long term field data coupled with a computer simulation model. I focus on the obligate brood parasitic brown-headed cowbird (*Molothrus ater*) for which the farming hypothesis was first proposed (Arcese et al. 1996). I quantify cowbird attacks on the nests of their song sparrow (*Melospiza melodia*) hosts using video recordings and suggest a reliable and conservative predator identification key that resolves whether a cowbird or conventional predator

was responsible for an attack based on the condition of the failed nest. I test the broad prediction of the farming hypothesis that the overall rate of survival is higher in parasitized than non-parasitized nests. I also directly test whether cowbirds tend to attack disproportionately fewer parasitized nests using my predator identification key. I test whether parasitism is relatively high in the re-nesting attempts following a cowbird attack, which would suggest that cowbirds actually benefit by taking advantage of the reproductive opportunities they create. Lastly, I ran a series of Monte Carlo simulations estimating the relative number of successful offspring of non-, simple and advanced farmers under different ecological conditions in order to consider the possible benefits of a farming strategy.

2.2 Methods

2.2.1 Study site and field methods

I studied nest predation and cowbird parasitism on two populations of song sparrow hosts over nine years near Victoria, British Columbia (Rithet's Bog Conservation Area: 44ha, 2000-2007, 2009-2010; Swan Lake Nature Sanctuary: 42ha, 2000-2003, 2010). I define nest predation by cowbirds as the killing without consumption of eggs and/or nestlings. These sites contain small to medium sized ponds, dense willow thickets (*Salix spp*., 1-4 m tall), and grassy fens. Song sparrows in this area are multi-brooded rearing up to four broods with 1-4 young per year (Zanette et al. 2006). In my study sites song sparrows are year-round residents whereas cowbirds are migratory and the arrival of cowbirds occurs after the start of song sparrow breeding (Zanette et al. 2003). Breeding begins in late March or early April and ends in late July. Hosts were

captured using mist nets and uniquely colour-banded for individual identification. Migratory cowbirds arrive to the area and begin parasitizing in late April (mean ± SE = 28 April ±2.7 days) and lay their last egg by mid-July (mean ± SE = 8 July ± 1.6 days).

Song sparrow nests were found using behavioural cues from the parents and were checked upon every 1-4 days until the nest either failed or fledged. Age of the nest was determined by candling eggs or back-dating from hatch. Nests that had already failed or fledged prior to the seasonal arrival of cowbirds were not considered in this study. A total of 741 nests were monitored for which I could reliably determine their fate (failed/ fledged, parasitized/ non-parasitized). Parasitism was high in these study sites, with almost half of all sparrow nests on average containing at least one cowbird egg (i.e. parasitized) in a given year (mean ± SE = 48.3 ± 5.3%; range = 17-78%). During each check on a song sparrow nest we recorded the number of host and cowbird eggs/ chicks present in the nest and inspected eggs closely for puncture marks or other damage. Whenever possible, from 2004-2010, a small motion sensitive video camera was erected next to a nest in order to continuously monitor activity and identify potential nest predators (see Zanette, et al. 2011 for camera details). I could identify the fate (failed/ fledged) with certainty for a total of 89 nests monitored with cameras. These cameras were crucial to this study as they allowed me to identity the specific predatory species that was responsible for a given nest failure. However, cameras could only be positioned for nests that were no further than 100m from out two fixed digital video recorder locations, and could only be deployed nine days into incubation to avoid abandonment by the host. Therefore, for all non-camera nests I devised a simple key to identify the predator type responsible for a given nest failure.

2.2.2 Predator identification key

My predator identification key was based on the condition of the nest and allowed me to determine whether a cowbird or non-cowbird predator was responsible for a nest failure event even where the nest was not monitored by a camera (see Smith et al. 2003, Swan et al. 2015 for examples). My goal was to be as conservative as possible when judging whether an attack was by a cowbird or conventional predator. Using the video records and past studies as a frame of reference, I identified broad characteristics of failed nests that were unique to cowbird visits, unique to non-cowbird predators, or could have been caused by either a cowbird or conventional predator.

Cowbirds characteristically either removed all nest contents or left some punctured eggs or nestlings in or under the nest and were never observed to cause noticeable damage to the nest itself. No other predator was found to leave punctured eggs or nestlings with minor wounds, but non-cowbird predators did occasionally empty nest contents without damaging the nest itself. Consequently, to be as conservative as possible, I only considered that a cowbird attack was the cause of nest failure whenever at least one punctured egg or nestling was found in or around an undamaged nest or a researcher observed a cowbird attacking a nest either directly or indirectly using the video system. Under the farming hypothesis it is expected that the parasites will cause late-stage nests they have not previously parasitized to fail in order to create the opportunity to parasitize the replacement nests (Swan et al. 2015). As detailed in the Introduction, cowbirds occasionally remove eggs in conjunction with the laying of their egg in a nest (i.e. parasitism) and this reduction in clutch size or the addition of a cowbird egg may cause hosts to abandon their nests (Peer 2006). Such nest failures should not be considered within a farming context, but instead suggest failed attempts at parasitism. Cowbirds use both direct and indirect cues to assess

the age of host eggs (Swan et al. 2015), and as this of course entails some degree of error they may occasionally lay their eggs in unsuitable (e.g., late-stage) nests. Nest stage alone is thus not a sufficient criterion to judge whether a nest failure was attributable to infanticide, or a failed attempt at parasitism, so to be as conservative as possible, I accordingly excluded all nest failures that occurred within two days of a cowbird egg being laid in the nest, regardless of nest stage, in my comparisons of cowbird and conventional predator attacks.

I considered that a non-cowbird predator was responsible for nest failure whenever: nest contents were substantially damaged (i.e. egg fragments/ body parts were found in or around the nest); the nest lining was pulled up; a hole was present in the bottom of the nest; the nest was completely removed or missing or; a researcher observed the attack directly or indirectly. Clutches were considered abandoned (e.g. because the parent has been killed) when eggs were cold with no other signs of damage and no parents were observed near the nest for at least two visits over four days (Smith et al. 2003). Nests that I could not definitively assign to either a cowbird or non-cowbird predator or abandonment based on these criteria (i.e. nest undamaged with contents missing) were not assigned to either category and were excluded from any analyses directly comparing cowbird to non-cowbird attacks.

2.2.3. Statistical Analyses

All statistical analyses were performed using the R statistical software (R development team 2008). A key correlate of the farming hypothesis is that parasitized nests should survive at higher rates than non-parasitized nests because parasites should not attack nests they have already laid in (Arcese et al. 1996). I compared predation rates of parasitized vs unparasitized nests broadly by calculating Mayfield daily survival values (Davies and Sealy 1998) and using the Mann-

Whitney test. Hauber (2000) suggested that the presence of a parasitic nestling, unlike a parasitic egg, may bring about loud vocalizations and elicit increased activity around the nest by the host parents, ultimately attracting increased predation pressure on parasitized nests post-hatch (Broughton et al. 1987; Haskell 1994; Lichtenstein and Sealy 1998, Dearborn 1999). Therefore, similar to Hauber (2000) I compared daily survival values only up until hatching. In order to more directly assess whether cowbirds avoid attacking non-parasitized nests, I compared the number of parasitized and non-parasitized nests attacked by cowbirds and non-cowbird predators— as determined by my predator identification key— using a Fisher's exact test. Another central tenet of the farming hypothesis is that, in order for farming behaviour to persist across generations, one would expect that cowbirds will take advantage of the laying opportunities created by their predatory action. Accordingly, one would expect the re-nesting attempts following a cowbird attack to be parasitized at a disproportionately high rate. Using a Fisher's exact test, I compared the frequency of cowbird parasitism in the re-nesting attempts following cowbird attacks with those following attacks by non-cowbird predators— estimated using my predator identification key. In order to be included in this analysis re-nesting attempts must have been on the same host territory and involved the same host pair as the previous nest attacked.

2.2.4. Simulation model

If cowbirds selectively attack host nests found too late in the nesting cycle to be suitable for parasitism they theoretically can benefit by parasitizing the replacement nests. The actual benefits of adopting a farming strategy are difficult to assess, however, because field experiments comparing farmers and non-farmers are not feasible. For these reasons, I created a Monte Carlo simulation model (Appendix 2) using python programing language (Python

Software Foundation 2016) in order to evaluate: 1) whether attacking nests within a farming context would yield worthwhile reproductive gains and; 2) the level of behavioural sophistication necessary for farming to be a realistic strategy. For this model I define three types of hypothetical cowbird each with incremental levels of farming sophistication. First, the 'non-farmer' lays only in host nests that are at an appropriate stage for parasitism and disregards late-stage nests. Nests appropriate for parasitism are defined as those whose contents are early enough in embryonic development that a parasitic egg will receive adequate incubation to hatch. Second, the 'simple farmer' also lays in appropriate nests, but destroys any non-parasitized, late-stage nests they encounter, causing the hosts to commence a new nesting attempt. The simple farmer theoretically benefits by increasing the proportion of appropriate nests available at any given time. Third, the 'advanced farmer' acts just as the simple farmer with one important difference— they gain knowledge on the location and timing of any re-nesting attempts created by their destructive actions. The advanced farmer is therefore able to find and parasitize those re-nesting attempts with higher probability than they otherwise would. The purpose of the simulations was not necessarily to calculate realistic estimates of cowbird reproduction, but to visualize the conditions under which either farming strategy would be beneficial relative to a non-farmer in order to evaluate whether such tactics could actually develop and persist.

If we assume no cost to finding and attacking nests, both simple and advanced farmers would enjoy a greater theoretical reproductive output than a non-famer, because by inducing host re-nesting their actions make more host nests available to lay in, however, these relative benefits may vary depending on other ecological conditions. For these simulations I identify and vary three particular ecological parameters that would logically impact the viability of a farming strategy. First, the number of host pairs present may affect the need to farm. More hosts available

may negate the need to create additional parasitism opportunities by attacking late-stage nests. In my simulations I define *n* as the number of host pairs available to a cowbird. Second, the likelihood that individuals naturally discover nests could impact the necessity to farm hosts because conspicuous nests would be easily found. I define *d* as the daily probability a nest may be discovered by a cowbird in a simulation. Third, variation in background predation rates caused by non-cowbird predators could drastically alter the benefits of farming. Unlike predatory cowbirds, other predatory species indiscriminately attack non-parasitized and parasitized nests alike, reducing the overall number of cowbird offspring, but at the same time increasing the number of laying opportunities available. For the simulations, I define the daily probability that a nest will be attacked by a non-cowbird predator as *a*. I ran a series of Monte Carlo simulations where we varied *n*, *d*, and *a* in order to visualize the reproductive output of my three hypothetical cowbirds (non-farmer, simple, and advanced farmer). Other than *n*, *d*, and *a* all other parameters in the simulations were constant values appropriate to my sparrow-cowbird system, however, the conditions of the simulation could easily be altered to apply to other host-parasite systems.

The hosts in the simulations behave as follows. A 120 day breeding season begins with *n* host pairs. On day one each host pair initiates a nest which goes through the following sequential developmental stages over subsequent days: building (4 days), laying (3 days), early incubation (4 days), late incubation (10 days), brooding (10 days). This developmental progression mimics what exists in my field sites. Song sparrows typically spend 3-4 days building a nest, lay 3-4 eggs and begin incubation after laying the penultimate egg. They incubate for 13 days before the clutch hatches after which they brood nestlings for approximately 10 days. I assume that nests are only appropriate for parasitism during the laying and early incubation stages of development. Each nest has the daily probability *a* of being attacked by a

non-cowbird predator thereby simulating background predation levels. The likelihood that a nest will fail because of a non-cowbird predator is unaffected by the parasitism status (y or n) of a nest, the number of other nests that were attacked on a given day, or the developmental stage of the nest. Following an attack, the nest will be considered a failure and the host pair begins building a new nesting attempt following a four-day idle period. Whenever a nest survives past brooding it is considered a success and the host pair also begins building a new nesting attempt after a four-day idle period.

Cowbirds in the simulations behave in the following way. I assume only one cowbird exists in each simulation and they are able to lay an egg every second day. Ovarian dissections of wild breeding birds estimate the potential laying rates of cowbirds to be between 0.5 and 0.8 eggs per day (Payne 1976, Scott and Ankney 1983, Rothstein et al 1986). However, it is worth noting that this potential is almost certainly less than the realized fecundity because cowbirds will often not find an adequate nest in which to lay (e.g. Woolfenden et al. 2001). On any day a cowbird may discover each nest with equal probability *d*. No action is taken if a cowbird discovers a nest during the building stage. If the nest is discovered at an appropriate stage for parasitism (laying/ early incubation) and the cowbird has an egg ready it will lay in (i.e. parasitize) that nest regardless of the type of cowbird (non-farmer, simple or advanced farmer). Upon discovering nests no longer appropriate for parasitism, non-farmers take no action. Simple and advanced farmers, meanwhile, will attack late-stage nests causing those hosts to begin building a new nest following a four-day idle period. Unlike non-cowbird predators, neither type of farmer will attack nests that they have previously parasitized. Following an attack by a simple farmer, the probability of encountering future nests will remain unchanged. When advanced farmers cause nests to fail on the other hand they improve their daily probability of discovering a

nest (*d*). Specifically, replacement nests following an attack by an advanced farmer will be found with a probability of 100%. The simulations assume that cowbirds can only perform one action per day. That is, in a given day, they may parasitize or attack, but may not do both and may not attack multiple nests. A single simulation runs until the end of the breeding season at which time the number of successful and failed parasitized and non-parasitized nests are recorded.

For all three types of cowbird, the simulation was run 10 000 times using levels of *n*, *d* and *a* that could realistically occur in the field. The number of host nests available ranged from 5 to 25. Genetic analyses of eggs collected from host nests reveals that individual cowbirds may parasitize between two and 12 separate hosts during a field season (Alderson et al. 1999; Ellison et al. 2006; de la Colina et al. 2016; Chapter 4). Field censuses estimate as few as 0.5 and as many as 14 song sparrow pairs per hectare (Arcese et al. 1992). I varied the daily probability of a cowbird discovering a nest (*d*) from 0.05 to 0.25 reflecting the extremes that were observed in the historic parasitism rates in my study sites. Over a seven-day parasitism period (laying/ early incubation) one can extrapolate the total probability a nest will be parasitized to be between 30 and 86%. I varied the daily probability that a nest will be attacked by a non-cowbird (*a*) to reflect the absolute extremes that hosts may experience in the field. Values of *a* from 0.01 and 0.10 predict that the total survival probability over a 26 day nesting cycle would be between .07 and 0.77.

In order to visualize the relative reproductive benefit of either type of farming strategy, I calculated the ratio of cowbird chicks fledged for each type of farmer to the number fledged by the non-farming cowbird. I then calculated and report the mean and standard deviation of these ratios. Because I am reporting ratios, a value of one indicates that the number of chicks fledged was the same as a non-farmer while values greater than one indicate that more chicks fledged

than the non-farmer. Note that by design, these simulations result in the absolute number of chicks fledged for all three types of hypothetical cowbirds to increase with the number of hosts (*n*) and chance of discovery (*d*) and to decrease with other predation pressure (*a*). Appendix 3 shows the absolute number of nests parasitized, cowbird attacks, and cowbird chicks fledged within the simulations as a frame of reference. I reiterate that the goal was not to predict actual reproduction in the field, but to consider the ecological situations where attacking nests within the context of the farming hypothesis would actually prove beneficial relative to a non-predatory individual.

2.3 Results

I found strong evidence that cowbirds regularly commit infanticide, destroying all nest contents in late-stage nests. Indeed, cowbirds destroyed more nests than any of the seven species of non-cowbird predators identified on camera, cowbirds being responsible for 20 (38.5%) of 52 nest failures followed most closely by cats, *Felis catis*: 22.9%; then rats, *Rattus norvegicus*: 15.4%; snakes, *Thamnophis spp*: 9.6%; raccoons, *Procyon lotor*: 7.7%; mink, *Mustela vison*: 5.8%; crows, *Corvus brachyrhynchos*: 5.8%; and Virginia rails, *Rallus limicola*: 2%. My predator identification key yielded very similar results. Of the 286 nest failures for which I could reliably assign a predator, I estimated that 112 (39.2%) failed as a result of an interaction with a cowbird and 174 (60.8%) were caused by a non-cowbird predator. The great majority (80%) of video recorded nest failures caused by cowbirds were what would be expected under the farming hypothesis as they involved the partial or total destruction of nest contents without egg laying. Video-recording verified that cowbirds are not perfect at estimating the age of host nests,

because in four cases the cowbird laid an egg in the nest in association with causing nest failure, even though all of the nests filmed were at too late a stage in development to be suitable to lay in. Considering cowbird related failures identified using my key, and focusing only on later-stage nests (i.e., those unsuitable to lay in; N = 56) I found results directly comparable to those observed with the cameras, the great majority (82%) of nest failures judged caused by cowbirds evidently being instances of infanticide (farming), because they were not associated with egg-laying by the cowbird.

Consistent with the prediction from the farming hypothesis that cowbirds avoid attacking nests they have already laid in I found evidence that parasitized nests survive at higher rates than non-parasitized nests. I found that Mayfield daily survival rates until hatching were significantly higher for parasitized than non-parasitized nests ($P = 0.022$; Figure 2.1). Furthermore, I found that cowbirds caused disproportionately fewer parasitized nests to fail compared to non-cowbird predators. According to my predator identification key, I found that only 27% (N = 55) of nests attacked by cowbirds contained a cowbird egg at the time of attack while 42% (N = 156) of nests attacked by non-cowbird predators were previously parasitized (Fisher's exact test, P = 0.054, Figure 2.2).

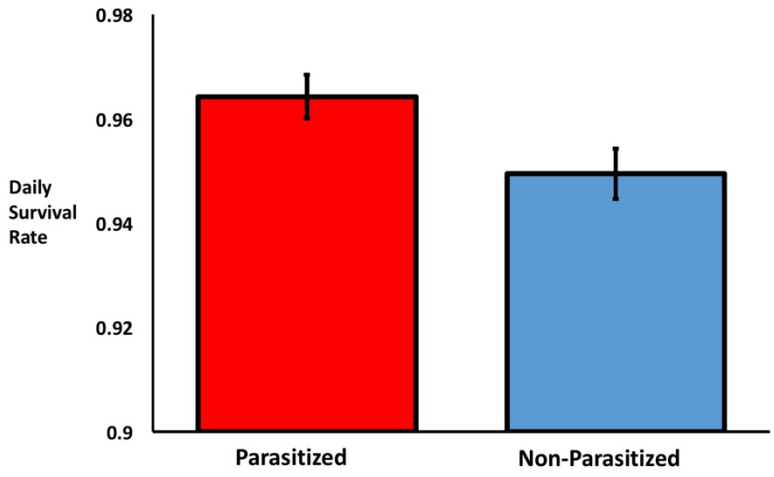

Figure 2.1: Mayfield's daily survival rates for parasitized and non-parasitized nests. Error bars indicate Standard Deviation.

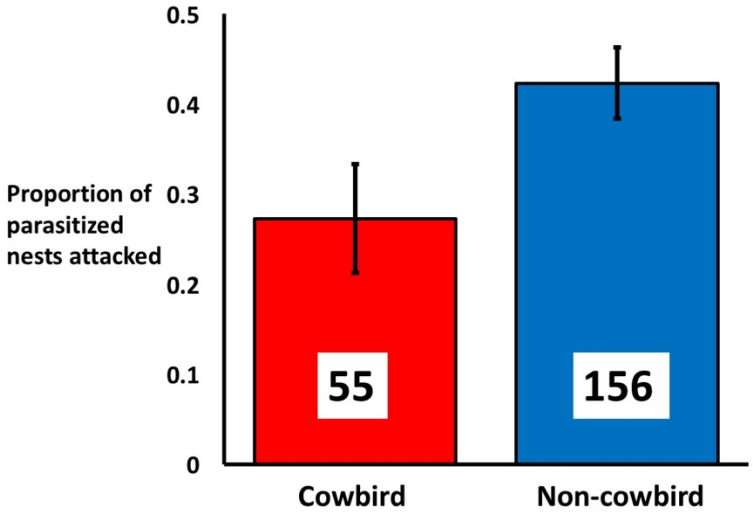

Figure 2.2: The proportion of nests attacked by cowbirds and non-cowbirds that were parasitized at the time of attack. Numbers inside bars indicate sample size. Error bars indicate Standard Error.

As expected if cowbirds destroy nests to improve their future parasitism opportunities, I found evidence that the re-nesting attempts of sparrows that had their nest destroyed by a cowbird were parasitized at relatively high rates. I was able to determine the parasitism state (yes or no) of the re-nesting attempts following 42 cowbird and 108 non-cowbird predator attacks. A Fisher's exact test revealed that the re-nesting attempts following a cowbird attack were parasitized significantly more frequently (59.5%) than the nests following an attack by a non-cowbird predator (39.8%, P = 0.043, Figure 2.3).

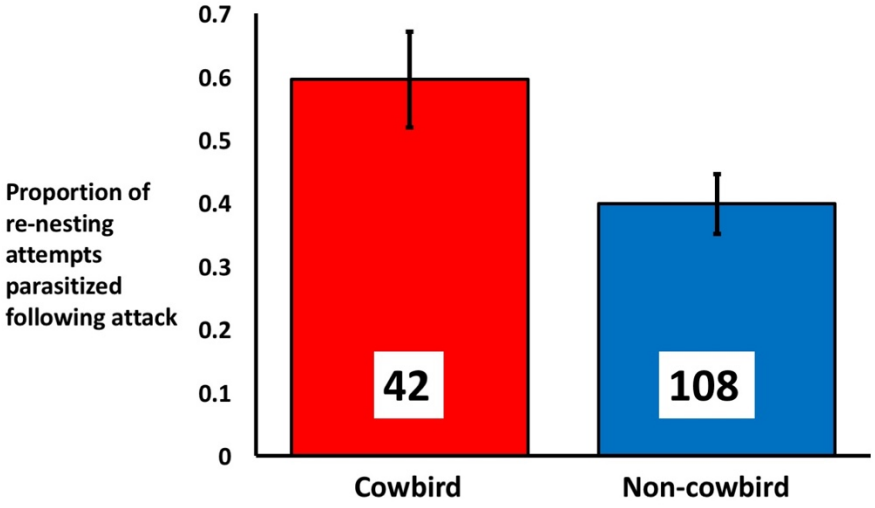

Figure 2.3: The proportion of nests attacked by cowbirds and non-cowbirds that were parasitized at the time of attack. Numbers inside bars indicate sample size. Error bars indicate Standard Error.

Figure 2.4 shows the results of my Monte Carlo simulations estimating the reproductive output of my two hypothetical cowbird farmers (simple, advanced) in relation to that of the non-farming cowbird. The relative number of chicks fledged is presented as a function of *a* (daily probability of attack by a non-cowbird predator) using four illustrative combinations of *d* (daily probability of discovery by a cowbird) and *n* (number of hosts available) in order to visualize the relative benefit of either farming strategy under different ecological conditions. As a reference, Appendix A shows the actual estimates of the number of chicks fledged for each cowbird type. The simulations suggest that a simple farming strategy, where individuals destroy late-stage nests with no increase in the likelihood of finding the subsequent replacement nest, would rarely provide adequate reproductive advantage to be worthwhile. Except in cases where predation levels are unusually low and nest discovery is high, the number of chicks fledged by the simple farmer does not appear substantially different from that of the non-farmer ($a = 0.01$, $d = 0.25$, Figure 2.4c-d). Yet, an advanced farming strategy, where attacking a nest increases the probability of finding the replacement nest, appears advantageous under most conditions. In particular, advanced farmers in the simulations enjoy a two-to-three-fold reproductive advantage over non-farmers when nests are difficult to discover and predation levels are low ($a = 0.01$-0.05, $d = 0.05$, Figure 2.4a-b). This relative advantage diminishes, however, as nests become easier to discover ($d = 0.25$, Figure 2.4c-d) suggesting that farming is unnecessary when nests will be found regardless. Generally, the relative benefit of a farming strategy decreases as background predation levels (*a*) increase, likely because predators provide the same function as a farming cowbird by creating more nests available to be parasitized while simultaneously causing greater failure among non-parasitized and parasitized nests alike. Although, increasing the number of hosts available (*n*) increases the absolute number of chicks fledged by any cowbird, it appears to

have little effect on the relative reproductive benefit of a farming cowbird to that of a non-farmer (Figure 2.4a vs b and Figure 2.4c vs d).

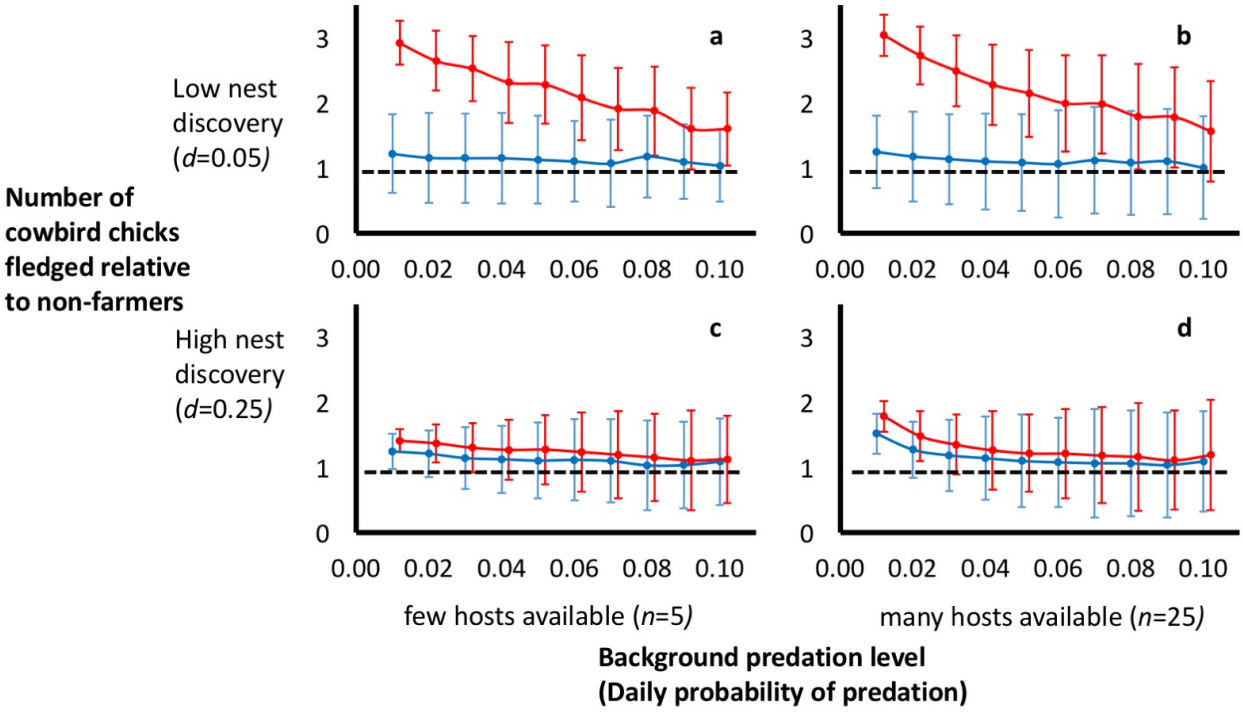

Figure 2.4: The proportion of cowbird chicks fledged by simple (blue) and advanced farmers (red) to that of non-farming cowbirds. Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*). Values above the dotted line are greater than that of non-farmers.

2.4 Discussion

These results satisfy several of the fundamental requirements of the farming hypothesis and coincide with evidence from other systems that show infanticide may yield reproductive benefits. I found ample evidence from the camera data and predator identification key that brown-headed cowbirds meet the minimum requirement of a farming strategy— namely, that they cause the nests of their hosts to fail independent of laying an egg. Following the expectation of the farming hypothesis, parasitized nests in my study sites survived at higher rates than non-parasitized nests. Moreover, cowbirds appeared to attack disproportionately fewer parasitized nests than what would be expected from the attacks of conventional predators. My finding that replacement nests of hosts that lost their nest to a cowbird attack were parasitized at relatively high rates (59.5%) compared to other nests (39.5%) supports the critical tenet of the farming hypothesis that individuals gain reproductive benefit from their predatory actions. The Monte Carlo simulations suggest that attacking late-stage nests to simply increase the number of early stage nests available may not be enough to provide substantial reproductive benefit to a cowbird. Indeed, the simple farmer in the simulations rarely showed much of a reproductive gain relative to that of a non-farmer. Meanwhile, the advanced farmer appeared to fledge considerably more offspring than the non-farmer suggesting that more benefit is derived from increasing the discovery of replacement nests following cowbird attacks.

Our camera data represent the largest collection of cowbird attacks captured on video in any one study. Several past studies document personal observations or video recordings of cowbirds attacking host nests (DuBois 1956, Beane and Alford 1990, Sealy, 1994, Scott et al. 1992, Stake and Cimprich 2003, Small 2005, Elliot 1999, Thompson 1999), however, even in instances where host nests were video-recorded sample sizes were small (Granfors et al. 2001). I

was able to show that nearly forty percent of recorded nest failures were due to a cowbird. Estimates using my predator identification key mirrors this percentage almost exactly, especially when we focus on late-stage nests, validating my key as a reliable method of quantifying cowbird attacks and strongly implicating possible farming behaviour as a regular occurrence. I emphasize that my key is highly conservative because it excludes ambiguous failures and therefore almost certainly underestimates the actual number of cowbird attacks within my study area. The basic premise of the farming hypothesis depends primarily on establishing that infanticide by brood parasites is not uncommon, but an ordinary aspect of a parasitic lifestyle, in the same way that establishing that the killing of cubs by male lions was not an anomaly but a regular occurrence (Bertram 1975; Packer and Pusey 1984) was the first step in demonstrating sexually selected infanticide in lions.

I found that the Mayfield daily survival rates in my study sites were higher for parasitized versus non-parasitized nests. These results are consistent with the prediction that cowbirds should focus their attacks on nests that they have not already laid in and are corroborated by multiple studies (Arcese et al. 1996, Clotfelter and Yasukawa 1999, Hauber 2000), however, others report no such trend (Rothstein 1975, Kus 1999, Whitfield and Sogge 1999). High background levels of predation can potentially impede statistical detection of differences in survival rates of parasitized and non-parasitized nests, especially with lower sample size. This may partially explain why parasitized nests appear to fare significantly better in areas with naturally low predation or where predators were removed or otherwise prevented for accessing the nest (Smith and Arcese 1994, Arcese et al. 1996; Hauber 2000, Hoover and Robinson 2007). Corroborating this is the pattern that cowbird attacks happen on proportionately fewer parasitized nests than attacks by conventional predators— as estimated from my predator

identification key. But why do we find evidence that cowbirds attack any parasitized nests at all? In areas of overlap among individual laying ranges, cowbirds may destroy the contents of nests that their neighbour had already parasitized (Arcese et al. 1996). Unlike in my idealized Monte Carlo simulations, cowbird females do appear to have some degree of overlap among their breeding ranges (Chapter 4). Just as DNA evidence confirms that lions only attack the cubs of their rivals (Gilbert et al. 1991), future studies should determine whether cowbirds only attack parasitized nests when they contain a neighbour's egg and not their own.

According to the farming hypothesis, causing a nest to fail is adaptive only if the culprit takes advantage of the laying opportunity they create in the subsequent re-nesting attempt. Consistent with this prediction is my finding that the re-nesting attempts following cowbird attacks were parasitized at a disproportionately high rate (59.5%) compared to the re-nesting attempts following attacks by non-cowbird predators (39.8%). Similar results were reported by Hoover and Robinson (2007) for prothonotary warblers (*Protonotaria citrea*). While these results strongly support the farming hypothesis, I suggest that future studies confirm that individual females parasitize the re-nesting attempts caused by their own predatory behaviour, just as DNA evidence confirms that infanticidal male lions impregnate the mothers of the cubs they kill (Gilbert et al. 1991).

Overall, my Monte Carlo simulations suggest that simply creating additional laying opportunities is insufficient to make farming behaviour worthwhile. Indeed, simple farmers in the simulations fledged similar numbers of offspring as non-farmers. Instead, it appears that farming behaviour may yield substantial reproductive gains when it increases the chances of future nest discovery, as evidenced by the relatively high number of offspring fledged by advanced farmers. This would require that female parasites learn the approximate timing and

location of replacement nests following an attack and adapt their nest searching accordingly. Female cowbirds have relatively enlarged brain regions associated with spatial memory (Sherry et al. 1993; Reboreda et al. 1996) and perform well in spatial memory tasks (Guigueno et al. 2014) suggesting that they may be suited for such cognitive demands. Whether, such advanced nest searching behaviours manifest in the wild should be the subject of future research. The simulations further predict that the benefits of farming one's host may vary depending on ecological conditions. First, when nests are naturally easy to discover the relative benefits of farming are diminished. This is not entirely surprising because tactics to increase nest discovery would likely be redundant when nests will be found anyway. Second, the reproductive output of farmers approaches that of non-farmers as background levels of predation increase predicting that farming may be ineffective in areas with high nest predation. Conventional predators indiscriminately attack nests regardless of parasitism status and, when almost all nests will fail as a result, farming tactics may indeed prove futile. This corroborates my suggestion that differences in survival between parasitized and non-parasitized nests are difficult to detect where background rates of predation are high. Third, the relative benefits of farming were surprisingly unaffected by the number of hosts available. This reinforces my inference that increasing nest discovery is the true purpose of cowbird predatory behaviour.

Our results indicate that, as in intra-specific infanticide: 1) incidents of infanticide are not anomalous but occur quite commonly; 2) incidents of infanticide are not indiscriminate and culprits evidently avoid killing their own young; and 3) infanticide does appear to accelerate the rate at which reproductive opportunities become available. What remains to be shown is that cowbirds definitively do take advantage of the opportunities created by infanticide, using DNA evidence, just as DNA evidence confirms that infanticidal male lions impregnate the mothers of

the cubs they kill. Until such time as such DNA evidence becomes available it is perhaps premature to consider re-naming cowbirds “lionbirds”, but I suggest that my results strongly support there being a remarkable convergence between the reproductive strategies of avian brood parasites and the sexually selected strategies of infanticidal males.

2.5 References

Agoramoorthy G, Rudran R. 1995. Infanticide by adult and subadult males in free-ranging red howler monkeys, *Alouatta seniculus*, in Venezuela. Ethology 99: 75–88.

Alderson GW, Gibbs HL and Sealy SG. 1999. Determining the reproductive behaviour of individual brown-headed cowbirds using microsatellite DNA markers. Anim Behav 58:895-905.

Arcese P and Smith JNM. 1999. Impacts of nest depredation and brood parasitism on the productivity of North American passerines. In Adams N and Slotow R, editors, Proceedings of the 22 International Ornithological Congress. BirdLife South Africa, Durban. pp. 2953-2966.

Arcese P, Smith JMN, Hochachka WM, Rogers CM, Ludwig D. 1992. Stability, regulation, and the determination of abundance in an insular song sparrow population. Ecology. 73:805-822.

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. Proc Natl Acad Sci. U.S.A. 93:4608-4611.

Balda RP and Carothers S. 1968. Nest protection by the Brown-headed Cowbird (*Molothrus ater*). Auk 85:324-325.

Beane JC and Alford SL. 1990. Destruction of a Pine Warbler brood by an adult cowbird. Chat 54:85-87.

Bertram BC. 1975. Social factors influencing reproduction in wild lions. J Zool 177: 463-482.

Briskie JV. 2007. Direct observations of shining cuckoos (*Chrysococcyx lucidus*) parasitizing and depredating grey warbler (*Gerygone igata*) nests. Notornis. 54:15-19.

Broughton KE, Middleton ALA, and Bailey ED. 1987. Early vocalizations of the Brown-headed Cowbirds and three host species. Bird Behav. 7:27-30.

Chakra MA, Hilbe C and Traulsen A. 2014. Plastic behaviors in hosts promote the emergence of retaliatory parasites. Nature Scientific Reports 4, Article number 4251.

Clotfelter ED, Yasukawa K. 1999. Impact of brood parasitism by brown-headed cowbirds on red-winged blackbird reproductive success. Condor. 101:105-114.

Clotfelter ED. 1998. What cues do brown-headed cowbirds use to locate red-winged blackbird host nests? Anim Behav. 55:1181-1189.

Davies NB. 2000. Cuckoos, cowbirds and other cheats. London, UK: Oxford University Press.

Davies SK and Sealy SG. 1998. Nesting biology of the Baird's Sparrow in southwestern Manitoba. Wilson Bull. 110:262-270.

de la Colina MA, Hauber ME, Strusberger BM, Reboreda JC and Mahler B. 2016. Molecular tracking of individual host use in the shiny cowbird- a generalist brood parasite. Ecol. and Evol. 6:4684-4696.

Dearborn DC. 1996. Video documentation of a brown-headed cowbird nestling ejecting an indigo bunting from the nest. Condor 98:645-649.

Dearborn DC. 1999. Brown-headed cowbird nestling vocalizations and risk of nest predation. Auk. 116:448-457.

Donovan TM, Jones PW, Annand EM, and Thompson FR. 1997. Variation in local-scale edge effects: Mechanisms and landscape context. Ecology 78:2064-2075.

Dow D. 1972. The New Zealand long-tailed Cuckoo: nest parasite or predator? Emu. 72:179-180.

Dubina KM, Peer BD. 2013. Egg pecking and discrimination by female and male brown-headed bowbirds. J. Ornithol. 154:553-557.

DuBois AD. 1956. A cowbird incident. Auk 73:286.

Elliot PF. 1999. Killing of host nestlings by the Brown-headed Cowbird. J. Field Ornith. 70:55-57.

Ellison K, Sealy SG and Gibbs HL. 2006. Genetic elucidation of host use by individual sympatric bronzed cowbirds (*Molothrus aeneus*) and brown-headed cowbirds (*M. ater*). Can. J. Zool. 84:1269-1280.

Elwood RW and Kennedy HF. 1994. Selective allocation of parental and infanticidal responses in rodents: a review of mechanisms. In Parmigiani S and vom Saal F, editors, Infacnticide and Parental Care, Harwood, Chur, Switzerland. pp.397-425.

Fiorini VD, Tuero DT and Reboreda JC. 2009. Shiny cowbirds synchronize parasitism with host laying and puncture host eggs according to host characteristics. Anim Behav. 77:561-568.

Gilbert D, Packer C, Pusey AE, Stephens JC and O'Brien SJ. 1991. Analytical DNA fingerprinting in lions: parentage, genetic diversity and kinship. J. Heredity 82:378-386.

Gloag R, Fiorini VD, Reboreda JC, Kacelnik A. 2011. Brood parasite eggs enhance egg survivorship in a multiply parasitized host. Proc R Soc London B. 279:1831-1839.

Granfors DA, Pietz PJ, Joyal LA. 2001. Frequency of egg and nestling destruction by female brown-headed cowbirds at grassland nests. Auk. 188:765-769.

Guigueno MF, Snow DA, MacDuogall-Shackleton SA and Sherry D. 2014. Female cowbirds have more accurate spatial memories than males. Biol. Letters

Haskell D. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. Proc. Roy. Soc. Lond. B 257:161-164.

Hauber ME and Russo SA. 2000. Perch proximity correlates with higher rates of cowbird parasitism in ground nesting Song Sparrows. Wilson Bull. 112:150-153.

Hauber ME. 1998. Single-egg removal from an artificial nest by the Gray Catbird. Wilson Bull. 110:426-429.

Hauber ME. 2000. Nest predation and cowbird parasitism in Song Sparrows. J Field Ornithol. 71:389-398.

Hauber ME. 2014. Mafia or Farmer? Coevolutionary consequences of retaliation and farming as predatory strategies upon host nests by avian brood parasites.

Hitaiwa-Hasegawa M and Hiraiwa T. 1994. Infanticide in non-human primates: sexual selection and local resource competition. In, Parmigiani S and vom Saal FS, Infanticide and parental care, Switzerland: Harwood. pp. 137-154.

Hoogland JL. 2007. Alarm calling, multiple mating, and infanticide among black-tailed, Gunnison's, and Utah prairie dogs. In Wolff JO, Sherman PA, editors. Rodent societies: An ecological and evolutionary perspective. University of Chicago Press, Chicago. pp. 438-450

Hoover JP and Robinson SK. 2007. Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. Proc Nat Acad Sci U.S.A. 104:4479‑4483.

Hrdy SB. 1974. Male-male competition and infanticide among the langurs (*Presbytis entellus*) of Abu, Rajasthan. Folia. Primatol. 22: 19-58.

Hrdy SB. 1977. The langurs of Abu: Female and male strategies of reproduction. Harvard University Press, Cambridge, MA.

Kattan GH.1997. Shiny cowbirds follow the 'shotgun' strategy of brood parasitism. Anim. Behav. 53:647-654.

Kus B. 1999. Impacts of Brown-headed Cowbird parasitism on productivity of the endangered Least Bell's Vireo. Stud Avian Biol, 18:160-166.

Lichtenstein G and Sealy SG. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. Proc. Roy. Soc. B. 265:249-254.

Llambias PE, Ferretti V and Reboreda JC. 2006. Egg discrimination and sex-specific pecking behaviour in parasitic cowbirds. Ethology 112:1128-1135.

Lowther PE. 1993. Brown-headed cowbirds (*Molothrus ater*). In A Poole and F Gill eds, the birds of North America no 47. Philadelphia PA, American Ornithologists Union, Washington DC.

McLaren CM and Sealy SG. 2000. Are nest predation and brood parasitism correlated in yellow warblers? A test of the cowbird predation hypothesis. Auk. 117:1056-1060.

Ortega CP. 1998. Cowbirds and other brood parasites. The University of Arizona Press, Tucson, Arizona, U.S.A.

Packer C and Pusey AE. 1983. Male takeovers and female reproductive parameters: a simulation of oestrous synchrony in lions (*Panthera leo*). Anim. Behav. 31:334-340.

Packer C and Pusey AE. 1984. Infacnticide in carnivores. In G Hausfater and Hrdy SB, editors. Infanticide. Aldine, New York. pp31-42.

Packer C and Pusey AE. 1987. Intrasexual cooperation and the sex ratio in African lions. Am. Nat. 130:636-642.

Packer C, Herbst L, Pusey AEm Bygott JD, Cairns SJ, Hanby JP and Borgerhoff-Mulder M. 1988. Reproductive success in lions. In TH Chitton-Brock ed. Reproductive success. PP 363-383. University of Chicago Press, Chigago.

Pagel M, Møller AP and Pomiankowksi A. 1998. Reduced parasitism by retaliatory cuckoos selects for hosts that rear cuckoo nestlings. Behav. Ecol. 9:566-572.

Palombit RA. 2015. Infanticide as sexual conflict: Coevolution of males strategies and female counterstrategies. Cold Spring Harbour Perspective Biology 7:a017640.

Patterson IAP, Reid RJ, Wilson B, Grellier K, Ross HM and Thompson PM. 1998. Evidence for infanticide in bottlenose dolphins: an explaination for violent interactions with harbor porpoises? Proc. R. Soc. B. 265:1167-1170.

Payne RB. 1976. The clutch size and numbers of eggs of Brown-headed Cowbirds: effects of latitude and breeding season. Condor 78:337-342.

Payne RB. 1977. The ecology of brood parasitism in birds. Ann. Rev. Ecol. Syst. 8:1-28.

Peer BD and Bollinger EK. 2000. Why do female Brown-headed Cowbirds remove host eggs? A test of the incubation efficiency hypothesis. In: Ecology and management of cowbirds and their hosts: Austin USA: University of Texas Press. Pp. 187-192.

Peer BD, Rivers JW and Rothstein SI. 2013. Cowbirds, conservation, and coevolution: potential misconceptions and directions for future research Chinese Birds 4:15-30.

Peer BD. 2006. Egg destruction and egg removal by avian brood parasites: adaptiveness and consequences. Auk 123:16-22.

Pusey and Packer 1994. Infanticide in lions: consequences and counterstrategies. In Infanticide and parental care.

Python Software Foundation. 2016. Python language reference version 2.7. Available at http://www.python.org.

Reboreda JC, Clayton N and Kacelnik A. 1996. Species and sex differences in hippocampus size in parasitic and non-parasitic cowbirds. Neuroreport 7:505-508.

Robinson SK, Thompson FR, III, Donovan TM, Whitehead DR and Faaborg J. 1995. Science 267:1987-1990.

Rogers ME and Bates PA. 2007. *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. PLoS Pathog. 3:818-825.

Rothstein SI, Yokey DA and Fleischer RC. 1986. Social dominance, mating and spacing systems, female fecundity, and vocal dialects in captive and free-ranging Brown-headed Cowbirds. Current Ornithol. 3:127-184.

Rothstein SI. 1975. Evolutionary rates and host defenses against avian brood parasitism. American Naturalist 109:161-176.

Rothstein SI. 1982. Successes and failures in avian egg recognition with comments on the utility of optimality reasoning. Amer. Zool. 22:547-560.

Rothstein SI. 1990. A model system for coevolution: avian brood parasitism. Ann. Rev. Ecol. Syst. 21:481-508.

Schaller, G. B. 1972. The Serengeti lion. Chicago: University of Chicago Press.

Scott DM and Ankney CD. 1980. Fecundity of Brown-headed Cowbird in southern Ontario. Auk 97:677-683.

Scott DM and Ankney CD. 1983. The laying cycle of Brown-headed Cowbirds. Condor 94:579-584.

Scott DM, Weatherhead PJ and Ankney CD. 1992. Egg-eating by female Brown-headed Cowbirds. Condor 94:579-584.

Sealy SG. 1992. Removal of yellow warbler eggs in association with cowbird parasitism. Condor 94:40-54.

Sealy SG. 1994. Observed acts of egg destruction, egg removal, and predation on nests of passerine birds at Delta Marsh, Manitoba. Can. Field Nat. 108:41-51.

Sherman PW. 1981. Reproductive competition and infanti- cide in Belding's ground squirrels and other animals. In Alexander RD and Tinkle DW, editors. Natural selection and social behavior. Chiron, New York. pp. 311-331.

Sherry D., Forbes M., Khurgel M. and Ivy G. 1992. Females have a larger hippocampus than males in the brood-parasitic Brown-headed Cowbird. Proc. Nat. Acad. Sci. USA 90:7839-7843.

Small SL. 2005. Mortality factors and predators of Spotted Towhee nests in the Sacramento Valley, California. J. Field Ornithol. 76: 252-258.

Smith JNM and Arcese P. 1994. Brown-headed cowbirds and an island population of song sparrows: a 16 year study. Condor 96:916-934.

Smith JNM, Taitt MJ and Zanette L. 2002. Removing brown-headed cowbirds increases seasonal fecundity and population growth in song sparrows. Ecology. 83:3037-3047.

Smith JNM, Taitt MJ, Zanette L and Myers‐Smith IH. 2003. How do brown‐headed cowbirds (*Molothrus ater*) cause nest failures in song sparrows (*Melospiza melodia*)? A removal experiment. Auk. 120:772‐783.

Soler JJ, Sorci G, Soler M and Møller AP. 1999. Change in host rejection behavior mediated by the predatory behavior of its brood parasite. Behav. Ecol. 10:275-280.

Soler M, Soler JJ, Martinez JG, Møller AP. 1995. Magpie host manipulation by great spotted cuckoos: evidence for an avian mafia? Evolution. 49:770-775.

Stake MM and Cimprich DA. 2003. Using video to monitor predation at Black-capped Vireo nests. Condor 105: 348-357.

Stratford JA and Robinson WD. 2005. Gulliver travels to the fragmented tropics: geographic variation in the mechanisms of avian extinction. Front. Ecol. Environ. 3:91-98.

Stutchbury BJ. 1997. Effects of female cowbird removal on reproductive success of Hooded warblers. Wilson Bull. 109:74-81.

Sulc M, Procházka P, Capek M and Honza M. 2016. Birds use eggshell UV reflectance when recognizing non-mimetic parasitic eggs. Behav. Ecol. 27:677-684.

Swan DC, Zanette LY and Clinchy M. 2015. Brood parasities manipulate their hostsg: experimental evidence for the farming hypothesis. Anim. Behav. 105:29-35.

Tate J Jr. 1967. Cowbird removes warbler nestling from nest. Auk 84:422.

Tewksbury JJ, Hejl SJ and Martin TE. 1998. Breeding productivity does not decline with increasing fragmentation in a western landscape. Ecology 79:2890-2903.

Thompson FR, Dijal W and Burhans DE 1999. Video identification of predators at songbird nests in old fields. Auk 116:259-264.

Thompson FR. 2007. Factors affecting nest predation on forest songbirds in North America. Ibis 149:98-109.

Veiga JP. 2000. Infanticide by male birds. In van Schaik CP, Janson CH, editors. Infanticide by males and its implications. Cambridge University Press, Cambridge. pp.198–220.

Whitfield MJ and Sogge MK. 1999. Range-wide impact of brown-headed cowbird parasitism on the Southwestern willow fly-catcher (*Empidonax traillii extimus*). Stud Avian Biol, 18:182-190.

Woolfenden BE, Gibbs HL and Sealy SG. 2001. Demography of brown-headed cowbirds at Delta Marsh, Manitoba. Auk 118:156-166.

Zanette L, Clinchy M and Ha-Cheol S. 2009. Food-supplementing parents reduce their sons' song repertoire size. Proc. Roy. Soc. Biol. Sci. 276:2855-2860.

Zanette L, Clinchy M and Smith JNM. 2006. Food and predators affect egg production in song sparrows. Ecology. 87:2459-2467.

Zanette L, Haydon DT, Smith JNM, Taitt MJ and Clinchy M. 2007. Reassessing the cowbird threat. Auk. 124:210-223.

Zanette L, Smith JNM, van Oort H and Clinchy M. 2003. Synergistic effects of food and predators on annual reproductive success in song sparrows. Proc. Roy. Soc. London B. 270:799-803.

Zanette L, White AF, Allen MC and Clinchy M. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. Science. 334:1398-1401.

Chapter 3

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

3.1 Introduction

Parasites, of all sorts, exhibit a wide variety of strategies to maximize the successful transmission of themselves and/or their offspring, by exploiting typically unrelated hosts (Poulin 2007). One such strategy occurs when parasites manipulate host behaviour in a way that increases their probability of transmission to an uninfected host (Holmes & Bethel 1972; Poulin 2000). Examples of parasites altering host behaviour abound and the effects may be dramatic and often focus on host reproductive behaviour (Moore 2002). For instance, rodents infected with *Toxoplasma gondii* appear less 'fearful' of the parasite's definitive cat host (Berdoy et al. 2000) and may even seem more sexually attractive to uninfected members of the opposite sex (Vyas 2013). Host behavioural changes such as these may be considered an adaptive extended phenotype of the parasite (Dawkins 1982), when the behavioural change is actually caused by the parasite and can be shown to increase the probability of transmission (reviewed in Poulin 1995; Moore 2002; Thomas et al. 2005). Also critical is the need to identify the mechanisms involved in the behavioural change in order to understand the complexity and sophistication of the supposed manipulation (Nickol 2005; Thomas et al. 2005). Brood parasitic birds are functionally indistinguishable from conventional parasites and pathogens in that they may reduce

the reproductive success of infected hosts (Ortega 1998; Davies 2000; Hauber 2003; Kilner 2005; Smith et al. 2002) and their transmission success is wholly dependent on their ability to encounter hosts suitable for parasitism during the infective stage (i.e. during egg laying/ early incubation). Nevertheless, instances of adult brood parasites manipulating host behaviour in order to increase the probability of infection have rarely been considered (but see Soler et al. 1995; Ponton et al. 2006; Hoover & Robinson 2007).

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

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

Cowbirds typically cause nest failure by either puncturing or removing enough eggs to cause the host parents to abandon. Beyond farming, other hypotheses have been proposed to explain egg removal or egg puncture by cowbirds (Sealy 1992; Peer 2006; Hover & Robinson 2007). For example, female cowbirds are known to occasionally consume the eggs they destroy (Sealy 1992). Nutrition does not appear to be the primary motivator for attacking nests, however, as most documented incidents do not involve the cowbird eating any nest contents (Scott et al. 1992, Sealy 1992, Granfors et al. 2001). In cowbird parasitized nests, removal of host eggs may

enhance the incubation efficiency of the parasitic egg (incubation efficiency hypothesis: Peer & Bollinger 2000) or reduce future competition for a parasitic nestling (competition reduction hypothesis: Carter 1986; Llambias et al. 2006). Also in parasitized nests, if a host rejects the parasitic egg the parasite may retaliate by destroying enough of the nest contents to cause complete failure (mafia hypothesis: Hoover & Robinson 2007). The incubation efficiency, competition reduction and mafia hypotheses do not, however, explain attacks on non-parasitized nests, which are the focus of the farming hypothesis and this study.

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

3.2 Methods

3.2.1 Experimental Procedures

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

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

condition because eggs were occasionally found (approximately two per week) within the cages and males and females continued to perform breeding displays throughout the duration of the study.

Before each trial for each experiment, individual birds were transferred to an outdoor flight chamber and left for 24 hours to habituate. I randomly selected a subset of females from the captive population for each experiment and subjects used multiple times had a minimum five day interval between trials (18 ± 1.7 days, mean ± SE). No subject was used multiple times within a single experiment. Flight chambers contained natural perches situated across one wall along with food and water. Immediately before each trial, two artificial open-cup nests containing different egg stimuli (see below for details) were placed in either corner of the wall opposite the perches approximately one meter from the ground. A short perch was positioned proximate (within 10 cm) to each nest, to facilitate investigation. The nests were constructed from raffia and white glue shaped around a tennis ball. Trials commenced as soon as the researcher left the flight chamber and lasted for 20 minutes. All trials were video recorded in the absence of an observer. Generally I found that female cowbirds readily attacked experimental nests and usually punctured or removed their first egg within the first five minutes of a trial (199.3 ± 28.9 s, mean ± SE).

For each experiment I recorded and compared three possible measures of a cowbird's intent to cause a nest to fail. First, I compared the incidence of attack (i.e. total number of nests in which one or more eggs were destroyed) and the total number of visits (bird lands on nearby perch: following White et al. 2007) to each nest type. Second, I compared the overall attack intensity (proportion of eggs destroyed per nest) for each nest type. I reasoned that the proportion

of host eggs removed or punctured during an attack was a reasonable proxy for attack intensity and a bird's intent to cause failure (see field procedures for justification). Thirdly, I compared the number of incidents in which the entire nest contents were destroyed. This is the most unambiguous measure of intent to cause nest destruction, which is a necessary element of the 'farming' hypothesis.

3.2.2 Attacks and Developmental Stage

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

Eggs were collected from the nests of red-winged blackbirds (*Agelaius phoeniceus*) which are commonly parasitized by cowbirds and likely suffer from cowbird predation (Clotfelter and Yasukawa 1999). After collection, eggs were stored at 4°C for a minimum of 10 days, where as little as four hours of cooling would have ensured effective and humane

euthanization (Leary et al. 2013). All eggs were returned to ambient temperature prior to presentation.

Red-winged blackbirds are among the most abundant species of bird in North America (Yasukawa & Sercy 1995; Beletsky 1996) and are not protected under either federal or provincial law because they are considered agricultural pests (Migratory Birds Convention Act 1994). Also, the Canadian Council on Animal Care place eggs in the lowest category of invasiveness for wildlife studies (Category A: CCAC 2010). Even so, to minimize the impact on blackbird reproduction, I collected one egg per nest and only enough eggs to ensure a minimum sample size of 10 for this experiment (30 undeveloped, 30 developed eggs). Eggs left undamaged in trials were reused in subsequent trials so that I could maximize the total number of trials I could carry out without requiring collection of additional eggs (N = 26). In accordance with Canadian law and guidelines outlined by the Canadian Council for Animal Care these protocols were approved by the University of Western Ontario Animal Care Committee (Protocol Number: 2010-005).

3.2.3 Attacks and Egg Number

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

canary (*Serinus flaviventris)* eggs (21.1 x 14.3 mm), simulating a complete clutch, and a nest containing two canary eggs, simulating an early nest that is still in the egg laying stage (White et al. 2007; King 1979).

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

3.2.4. Attacks and Changing Number of Eggs

Cowbirds are known to evaluate a nest's suitability for parasitism by monitoring whether eggs appear across multiple days and perceive nests with an increasing clutch as being suitable for parasitism (White et al. 2009). This could allow females to better synchronize their laying with that of their hosts because a nest with a changing number of eggs would always be in the egg laying stage. A clutch size that could be indicative of completion of laying (i.e. which remains constant in size across days) meanwhile would simulate complete clutches where incubation has already commenced. To determine whether the rate at which eggs are added to a nest across days affects the readiness of female cowbirds to attack, I performed an experiment similar to that

described by White et al. (2009). Two nests were set up containing model plasticine eggs. Twenty females were presented with a nest containing two model eggs and a nest containing four model eggs. The initial setup mirrored experiment 1, but here each nest was wrapped in a protective ball (≈25 cm diameter) of 1.25 cm chicken wire mesh. This prevented the cowbirds from puncturing or removing any eggs, while still allowing them to approach and investigate. The following morning a third egg was added to the two-egg clutch. On the third morning a fourth egg was added to the three-egg clutch and the chicken-wire ball was removed from both nests. Thus, trials involved a choice between two nests containing four eggs each— one nest with a changing number of eggs and one with a constant clutch size.

3.2.5. Field Procedures

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

I monitored parasitism and predation on 1035 song sparrow nests *(Melospiza melodia*) resident near Victoria, British Columbia, Canada over 10 years (2000-2007; 2009-2010). Song sparrows make an ideal species for studying possible 'farming' behaviour as they are a favorite cowbird host (Woolfenden el al. 2003), do not reject cowbird eggs, and have been the subject of intensive study in the context of parasitism and predation in and around my study sites (Smith et al. 2002, 2003; Zanette et al. 2007; Zanette & Clinchy 2010). Song sparrows typically lay

clutches of 3-4 eggs and begin incubation after laying their penultimate eggs. A cowbird egg will only receive enough incubation to hatch if laid before day seven following the laying of the first sparrow egg (Zanette unpublished). Therefore nests were considered inappropriate for parasitism following this time. Other specific details regarding study sites, song sparrows and methods used to find and monitor nests can be found elsewhere (Zanette et al. 2003; Zanette et al. 2006). Migratory brown-headed cowbirds begin parasitizing nests in the area in late April (28 April ± 2.7 days, mean ± SE) and lay their last egg by mid July (8 July ± 1.6 days, mean ± SE). Discounting nests that failed or fledged before cowbirds arrived in the spring, 17 to 78% of nests were parasitized (contained at least 1 cowbird egg) in a given year (48.3 ± 5.3%, mean ± SE). Using behavioural cues from the parents (Zanette et al. 2006), nests were located and monitored regularly (every 1-4 days) until the nests failed or fledged. During each visit the number of host and cowbird eggs present in the nest was recorded. I candled eggs to determine age or back-dated from hatch day. During each visit the number of eggs found punctured or removed from the nest was also recorded. From 2004-2010 a total of 90 song sparrow nests in my study sites were monitored using a custom video surveillance system (see Zanette et al. 2011 for camera details). I found that 48 of these nests failed as a result of a predator attack and that cowbirds were the number one predator, responsible for 35% of all nest failures (Chapter 2). These video records show that cowbirds either removed all eggs or left punctured eggs in the nest. No other predator was found to leave punctured eggs in my study sites. I therefore reasoned that a cowbird had attacked a nest whenever: 1) one or more eggs were found punctured in or around the nest; 2) a researcher directly observed a cowbird attacking a nest; 3) a cowbird attack was captured on camera. Under these criteria, I estimate that 74 non-parasitized nests and 62 parasitized nests were attacked by a cowbird. This is likely a conservative estimate of the actual number of

cowbird attacks because I did not include nests where I could not definitively assign a cowbird as the predator (i.e. nest contents missing; 217 nests).

3.2.6 Statistical Analyses

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

3.3 Results

3.3.1 Attacks and Developmental Stage

Consistent with the prediction from the farming hypothesis that cowbirds preferentially cause late-stage nests to fail, cowbirds were more likely to destroy all three eggs when they attacked late-incubation stage nests and only one egg at the early-incubation stage (Fisher's exact test: P = 0.02). Of the 12 attacks on late-incubation stage nests 66%, 17% and 17% of cases involved the destruction of three, two and one egg respectively, whereas attacks on early-incubation stage nests showed the opposite pattern (0%, 11% and 89%, N = 9). Cowbirds destroyed a significantly greater proportion of eggs in nests containing developed late-incubation stage eggs than in nests containing undeveloped early-incubation stage eggs (Wilcoxon-signed ranks test: W = 76; P = 0.014, Figure 3.1). There was no significant difference in the overall incidence of attacks (Fisher's exact: early-incubation = 35%, late-incubation = 46%, P = 0.48) and cowbirds did not visit late-incubation (mean ± SE = 3.65 ± 1.24, median = 4, range = 0-20) and early-incubation stage (mean ± SE = 1.35 ± 0.21, median = 1, range = 0-3) nests a significantly different number of times (Wilcoxon-signed ranks test: W = 105, P = 0.16). I excluded nine of the 26 trials (35%) from analyses because these cowbirds did not destroy any eggs.

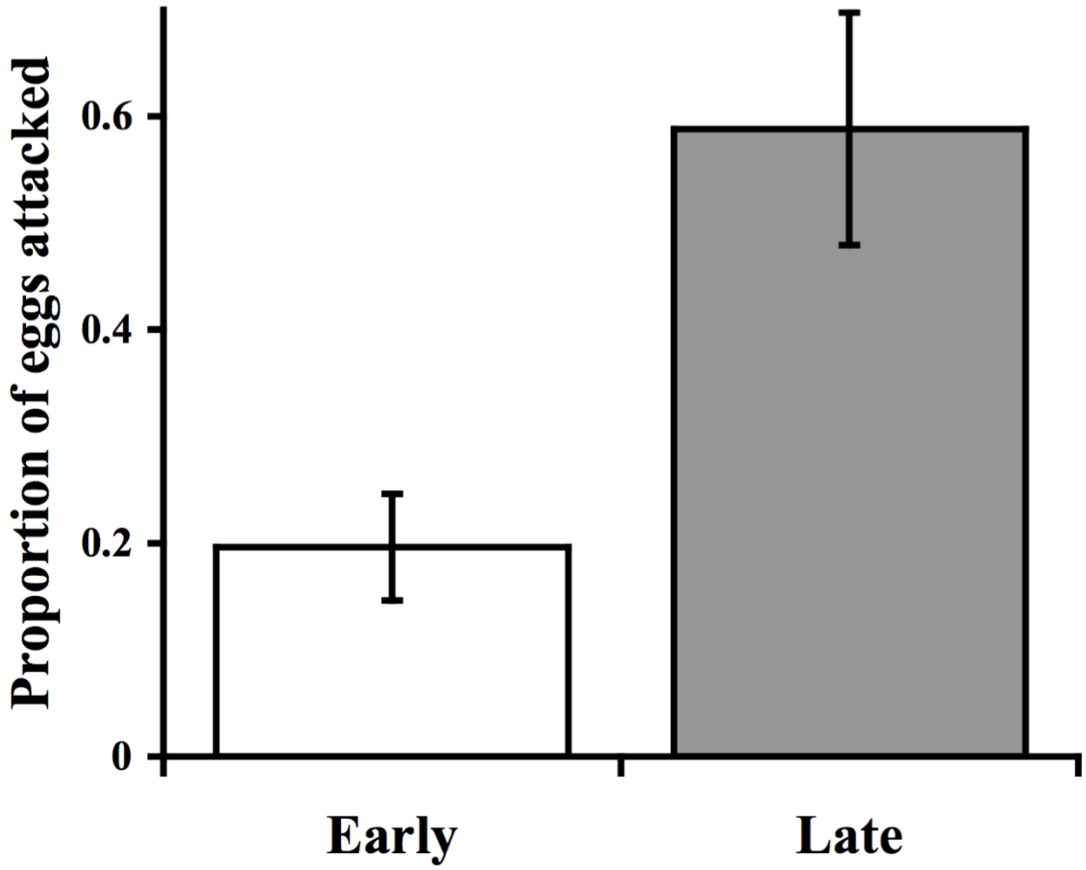

Figure 3.1: The proportion of eggs punctured or removed from experimental nests containing developed late-incubation eggs and nests containing undeveloped early-incubation stage eggs. Error bars indicate Standard Error.

3.3.2 Attacks and Egg Number

Consistent with cowbirds using absolute egg number to indirectly determine nest stage, I found that cowbirds destroyed over two times the proportion of eggs in four-egg nests than in two-egg nests ($W = 112.5$; $P = 0.0136$; Figure 3.2a). Out of the 15 four-egg nests attacked 40% suffered complete destruction and 27%, 20%, 13% had three, two and one eggs destroyed respectively. Attacks on two-egg nests meanwhile involved complete destruction 11% of the time and destruction of one egg 89% of the time ($N = 9$). Cowbirds did not attack nests containing four-eggs significantly more often than two-egg nests (four-egg = 58%; two-egg = 35%, $P = 0.164$). There was also no significant difference in the number of visits between the two nest types (two egg: mean ± SE = 3.76 ± 1.31, median = 2, range = 0- 36; four egg: mean ± SE = 2.00 ± 0.26, median = 1, range = 0-7 : $W = 298.5$; $P = 0.79$). Six of the 26 trials (23%) were excluded from analyses because no eggs were destroyed.

Corroborating that cowbirds attend to the absolute number of eggs in a clutch, when I increased the clutch size so that both treatments simulated complete clutches though a two-egg difference was maintained (four-eggs in one nest and six-eggs in the other), I found no significant difference in the proportion of eggs destroyed ($W = 130$; $P = 0.31$; Figure 3.2b). Moreover, both nests types were attacked an equal number of times (n = 15 of 26; $P = 1.00$) and visits to both nests were not significantly different (six-egg: mean ± SE = 3.47 ± 0.52, median = 3, range = 0-10; four-egg: mean ± SE = 3.84 ± 0.70, median = 4, range = 0-7; $W = 182.5$, $P = 0.96$). No eggs were destroyed in two out of 20 trials (10%) and were therefore excluded from analyses.

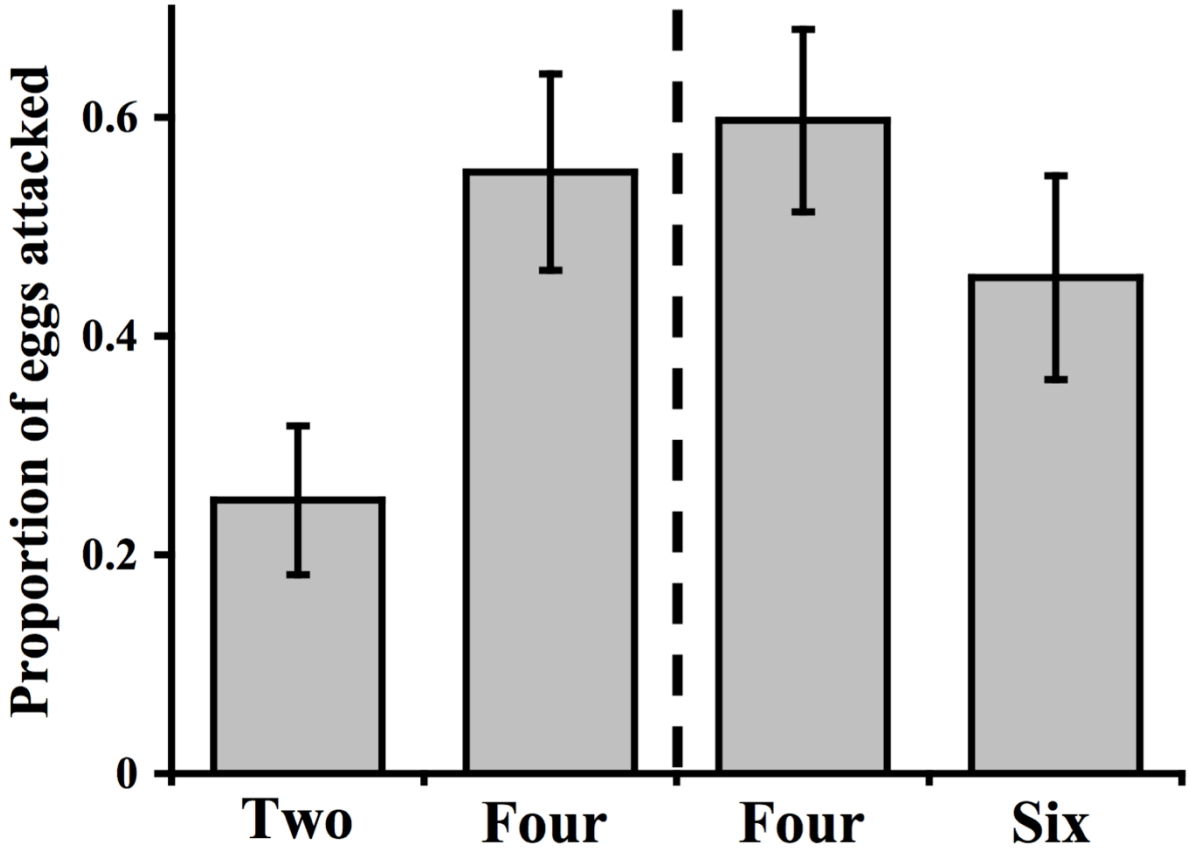

Figure 3.2: The proportion of eggs punctured or removed from experimental nests containing developed late-incubation eggs and nests containing (a) four-egg and two-egg clutches and (b) six-egg and four-egg clutches. Error bars indicate Standard Error.

3.3.3 Attacks and changing number of eggs

I found no evidence that cowbirds used the change in clutch size to indirectly evaluate nest stage. There was no significant difference in the proportion of eggs destroyed regardless of whether the clutch size changed on a daily basis or remained constant ($W = 99.5$; $P = 0.96$). Further, there was no difference in the number of attacks that resulted in complete destruction and those that

did not (P = 1.00). Attacks on nests with a changing clutch number resulted in four eggs, three eggs, two eggs and one egg destroyed 36%, 9%, 18% and 36% of the time. Attacks on nests with a constant clutch number ended in destruction of four, three, two and one eggs 30%, 50%, 0% and 20% of the time. There were also no significant differences between treatments in the overall incidence of attacks (constant = 50%, changing = 55%, P = 1.00), or the number of visits (constant: mean ± SE = 2.70 ± 0.744, median = 3, range = 0-14; changing: mean ± SE = 3.10 ± 0.864, median = 3, range = 0-11; W = 194.5; P = 0.89). Six out of 20 trials (30%) were omitted from analyses because no eggs were destroyed.

3.3.4 Wild Cowbird Predation

Based on my 10-year dataset concerning song sparrow nests in the wild, I found that cowbird attack effort increased significantly as the nesting cycle progressed on non-parasitized nests (Spearman: r_s = 0.35; $F_{1,72}$ = 11.08; P = 0.001; Figure 3.3), with no significant effect for parasitized nests (Spearman: r_s = 0.07; $F_{1,60}$ = 0.26; P = 0.61; Figure 3.4).

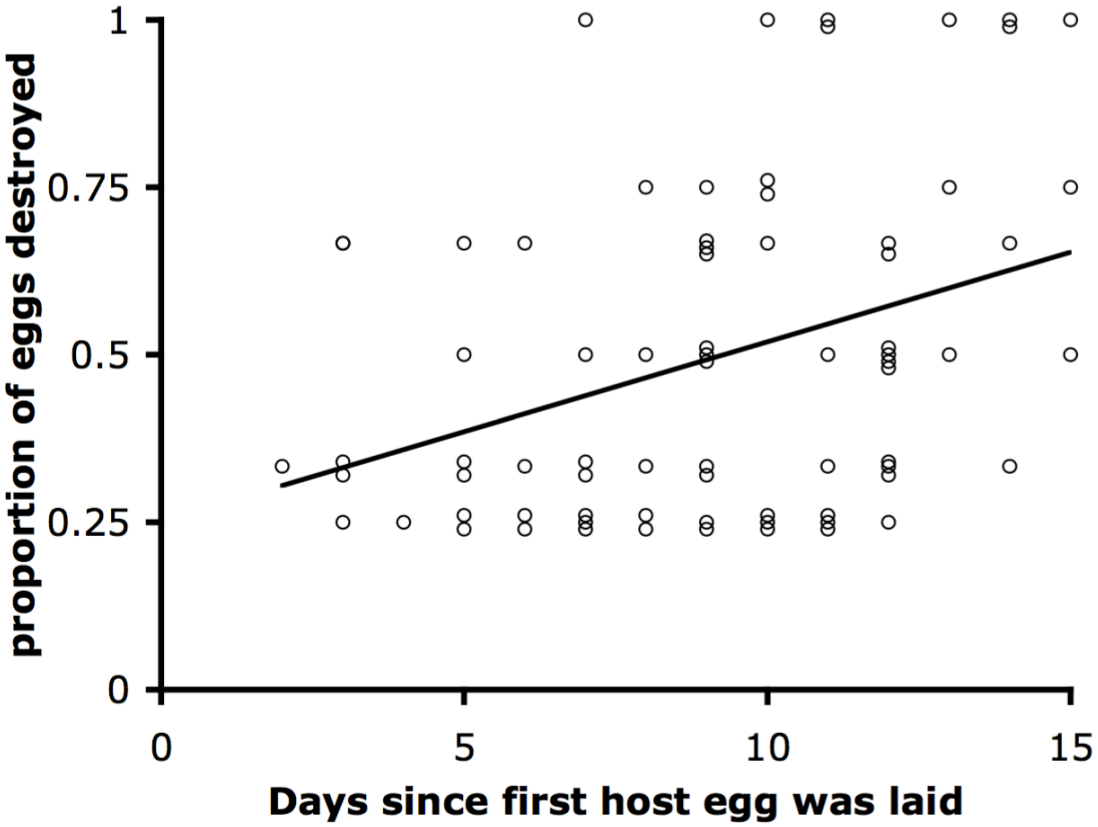

Figure 3.3: The proportion of eggs killed by cowbirds in non-parasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest (N = 74). Points are offset slightly where overlap occurs.

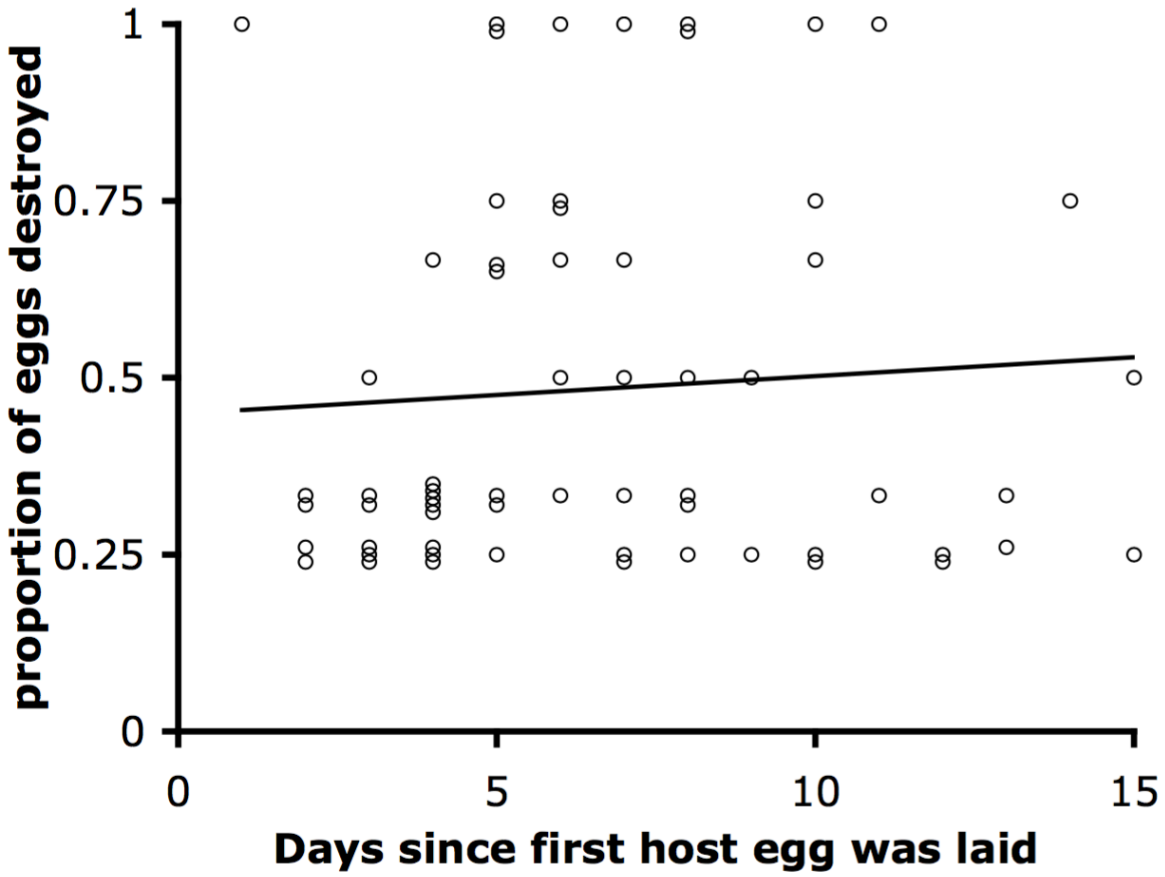

Figure 3.4: The proportion of eggs killed by cowbirds in parasitized nests relative to the day when the first host egg was laid. Each point represents one song sparrow nest (N = 64). Points are offset slightly where overlap occurs.

3.4 Discussion

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

The underlying mechanisms that I report in this study match some of the criteria necessary to conclude that 'farming' behaviour is adaptive for the parasite (Poulin 1995; Moore 2002; Thomas et al. 2005). Firstly, in order for 'farming' to enhance their probability of transmission, cowbirds would first need to direct intense attacks towards nests no longer suitable for parasitism, which is what out manipulations show. Secondly, host nests in the wild are more likely to fail as cowbird attack intensity increases, demonstrating that the hosts' behavioural change is unambiguously caused by the parasite. Lastly, my results suggest that cowbirds are capable of using both very direct and straightforward, and more indirect and thus, potentially more sophisticated mechanisms, to discriminate among nests. The fact that these parasites employ multiple methods of discrimination reinforces that 'farming' behaviour is not just a random act of violence, and instead could be an active attempt to manipulate their host's

reproductive behaviour. However, to complete the picture, one would need to demonstrate that 'farming' does improve the probability of transmission which could come about if the cowbird returns to parasitize the subsequent re-nesting attempts (Hoover and Robinson 2007) and/ or if cowbird-induced nest failure simply increases the proportion of suitable to unsuitable nests available for parasitism throughout the season. The relative benefits of either strategy should be the focus of future studies.

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

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

In my experiments I tested three methods by which cowbirds may assess host reproductive stage in relation to farming, based on established methods used in relation to parasitism (Massoni & Reboreda 1999; White et al. 2007; 2009). These are in no way the only methods cowbirds may use to evaluate nest stage and various authors have proposed a plethora of different means (e.g. egg temperature, host activity; Banks & Martin 2001). My lab results demonstrate that cowbirds evaluate nest stage by at least two means and my field results corroborate that they destroy nests depending on host reproductive stage. Which method they employ in the field will of course be governed by the set of cues available and likely requires substantial cognitive processing. My field and laboratory data were collected using different populations of cowbirds and, while not the focus of this research, future studies comparing nest

predation of different populations may yield valuable results. For example, 'farming behaviour' may become less necessary in areas where the density of host nests is high.

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

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

3.5 References

Alcock J. 2005. Animal Behavior, 8th edn., Sunderland, Massachusetts, USA: Sinauer Associates.

Arcese P, Smith JMN, Hochachka WM, Rogers CM, Ludwig D. 1992. Stability, regulation, and the determination of abundance in an insular song sparrow population. Ecology. 73:805-822.

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. Proc Natl Acad Sci. U.S.A. 93:4608-4611.

Banks AJ, Martin TE. 2001. Host activity and the risk of nest parasitism by brown-headed cowbirds. Behav Ecol. 12:31-40.

Beletsky LD. 1996. The red-winged blackbird: the biology of a strongly polygynous songbird. Academic Press, Sandiego, CA, U.S.A.

Berdoy M, Webster JP, Macdonald DW. 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. Proc R Soc Lond B. 267:1591-1594.

Canadian Council for Animal Care (CCAC) guidelines for: euthanasia of animals used in science. 2010. CCAC: Ottawa, ON, Canada.

Carter MD. 1986. The parasitic behavior of the bronzed cowbird *Molothrus aeneus* in South Texas USA. Condor. 88:11-25.

Clotfelter ED. 1998. What cures do brown-headed cowbirds use to locate red-winged blackbird host nests? Anim Behav. 55:1181-1189.

Clotfelter ED, Yasukawa K. 1999. Impact of brood parasitism by brown-headed cowbirds on red-winged blackbird reproductive success. Condor. 101:105-114.

Davies NB. 2000. Cuckoos, cowbirds and other cheats. London, UK: Oxford University Press.

Dawkins R. 1982. The extended phenotype. Oxford, UK: Oxford University Press.

Fiorini VD, Tuero DT, Reboreda JC. 2009. Shiny cowbirds synchronize parasitism with host laying and puncture host eggs according to host characteristics. Anim Behav. 77:561-568.

Friedmann H, Kiff LF, Rothstein SI. 1977. A further contribution to knowledge of the host relations of the parasitic cowbirds. Smithson Contrib Zool. 235:1–75.

Granfors DA, Pietz PJ, Joyal LA. 2001. Frequency of egg and nestling destruction by female brown-headed cowbirds at grassland nests. Auk. 188:765-769.

Hauber ME. 2003. Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. Behav Ecol. 14:227–235.

Holmes JC, Bethel WM. 1972. Modification of intermediate host behavior by parasites. In: Canning EU, Wright CA, editors. Behavioral Aspects of Parasite Transmission. London, UK: Academic Press, London. p. 123-149.

Hoover JP, and Robinson SK. 2007. Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. Proc Nat Acad Sci U.S.A. 104:4479-4483.

Hunt S, Low J, Burns KC. 2008. Adaptive numerical competency in a food-hoarding songbird. Proc R Soc Lond B. 275:2373–2379.

Kilner RM. 2005. The evolution of virulence in brood parasites. Ornithol Sci. 4:55-64.

Kilner RM, Madden JR, Hauber ME. 2004. Brood parasitic cowbird nestlings use host young to procure resources. Science. 305:877–879.

King AP. 1979. Variables affecting parasitism in the North American brown-headed cowbird (*Molothrus ater*). Ph.D. Thesis Cornell.

Krebs CJ. 2009. Ecology: The experimental analysis of distribution and abundance, 6th ed. San Francisco, USA: Benjamin Cummings.

Llambias PE, Ferretti V, Reboreda JC. 2006. Egg discrimination and sex-specific pecking behaviour in parasitic cowbirds. Ethology 112:1128-1135.

Lowther PE. 1993. Brown-headed cowbird (*Molothrus ater*). In: Poole A, Gill F, editors. The birds of North America, no. 47. Philadelphia, USA: American Ornithologists Union.

Leary S, Underwood W, Anthony R, et al. 2013. AVMA guidelines for the euthanasia of animals. Retrieved on 2014-12-24. <http://www.avma.org/kb/policies/documents/euthanasia.pdf>

Lyon BE. 2003. Egg recognition and counting reduce costs of avian conspecific brood parasitism. Nature. 422:495-499.

Massoni V, Reboreda JC. 1999. Egg puncture allows shiny cowbirds to assess host egg development and suitability for parasitism. Proc R Soc Lond B. 266:1871-1874.

Migratory Birds Convention Act. 1994. Retrieved on 2014-12-24. <https://www.ec.gc.ca/nature/default.asp?lang=En&n=496E2702-1>

Moore J. 2002. Parasites and the behavior of animals. Oxford, UK: Oxford University Press.

Nickol BB. 2005. Parasitic manipulation: should we go anywhere? Behav Process. 68:201-203.

Odell NS, Eadie JM. 2010. Do wood ducks use the quantity of eggs in a nest as a cue to the nest's value. Behav Ecol. 21:794-801.

Ortega CP. 1998. Cowbirds and other brood parasites. The University of Arizona Press, Tucson, Arizona, U.S.A.

Pagnucco K, Zanette L, Clinchy M, Leonard ML. 2008. Sheep in wolf's clothing: host nestling vocalizations resemble their cowbird competitor's. Proc R Soc Lond B. 275: 1061-1065.

Peer BD. 2000. Egg destruction and egg removal by avian brood parasites: adaptiveness and consequences. Auk. 123:16-22.

Peer BD, Bollinger EK. 2000. Why do female Brown-headed Cowbirds remove host eggs? A test of the incubation efficiency hypothesis. In: Ecology and management of cowbirds and their hosts: Austin USA: University of Texas Press. P. 187-192.

Peer BD, Sealy SG. 1999. Parasitism and egg puncture behavior by bronzed and brown-headed cowbirds in sympatry. Stud Avian Biol. 18:235-240.

Ponton F, Biron DG, Moore J, Møller AP, Thomas F. 2006. Facultative virulence: a strategy to manipulate host behaviour? Behav Process. 72:1-5.

Poulin R. 1995. "Adaptive" changes in the behaviour of parasitized animals: a critical review. J Parasitol. 25:1371-1383.

Poulin R. 2000. Manipulation of host behaviour by parasites: a weakening paradigm? Proc R Soc Lond B. 267:787–792.

Poulin R. 2007. Evolutionary ecology of parasites. Princeton, USA: Princeton University Press.

Scott DM, Weatherhead PJ, Ankney CD. 1992. Egg-eating by female brown-headed cowbirds. Condor. 94:579-584.

Sealy SG. 1992. Removal of yellow warbler eggs in association with cowbird parasitism. Condor. 94:40-54.

Smith JNM, Taitt MJ, Zanette L. 2002. Removing brown-headed cow- birds increases seasonal fecundity and population growth in song sparrows. Ecology. 83:3037–3047.

Smith JNM, Taitt MJ, Zanette L, Myers-Smith IH. 2003. How do brown-headed cowbirds (*Molothrus ater*) cause nest failures in song sparrows (*Melospiza melodia*)? A removal experiment. Auk. 120:772-783.

Soler M, Soler JJ, Martinez JG, Møller AP. 1995. Magpie host manipulation by great spotted cuckoos: evidence for an avian mafia? Evolution. 49:770-775.

Stoddard MC, Stevens M. 2010. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. Proc R Soc Lond B. 277:1387-1393.

Terres JK. 1980. The Audubon society of North American birds. New York, USA: Random House.

Thomas F, Adamo S, Moore J. 2005. Parasitic manipulation: where we are and where we should go? Behav Process. 68:185-199.

Trine CL. 2000. Effects of multiple parasitism on cowbird and wood thrush nesting success. In: Smith JNM, Cook TL, Rothstein SI, Robinson SK. Sealy SG, editors. Ecology and management of cowbirds: Austin USA: University of Texas Press. P. 135-144.

Vyas A. 2013. Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*. J Exper Biol. 216:120-126.

White DJ, Ho L, de los Santos G, Godoy I. 2007. An experimental test of preferences for nest contents in an obligate brood parasite, *Molothrus ater*. Behav Ecol. 18:922-928.

White DJ, Ho L, Freed-Brown G. 2009. Counting chicks before they hatch: Female cowbirds can time readiness of a host nest for parasitism. Psycol Sci. 20:1140-1145.

Woolfenden BE, Gibbs HL, Sealy SG, McMaster GD. 2003. Host use and fecundity of individual female brown-headed cowbirds. Anim Behav. 66:95-106.

Yasukawa K, Sercy W.A. 1995. Red-winged blackbird (*Agelaius phoeniceus*). In: Poole A, editor. The Birds of North America Online. Cornell Labd of Ornithology, Ithica, New York, U.S.A.

Zanette L, Smith JNM, van Oort H, Clinchy M. 2003. Synergistic effects of food and predators on annual reproductive success in song sparrows. Proc R Soc Lond B. 270:779-803.

Zanette L, Clinchy M, Smith JNM. 2006. Food and predators affect egg production in song sparrows. Ecology. 87:2459-2467.

Zanette L, Haydon DT, Smith JNM, Taitt MJ, Clinchy M. 2007. Reassessing the cowbird threat. Auk. 124:210-223.

Zanette L, Clinchy M. 2010. Food supplementation leads to bottom-up and top-down food-host-parasite interactions. J Anim Ecol. 79:1172-1180.

Zanette L, White AF, Allen MC, Clinchy M. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. Science. 334:1398-1401.

Chapter 4

4 Modelling breeding ranges in space and time: cowbirds follow available nests and avoid each other

4.1 Introduction

How animals occupy space within their environment is a primary focus of ecological and behavioural research and has direct implications for inter/intra-specific competition (e.g. Mattison et al. 2011), resource selection (Marzluff et al. 2004), and predator-prey/ parasite-host interactions (Millspaugh et al. 2000; Honza et al. 2002). Following the development of utilization distributions (UDs, Van Winkle 1975) our ability to understand space use of animals has moved beyond simple mapping of home range boundaries to establishing probabilistic measures of space use. Traditionally, UDs have been defined solely using horizontal spatial dimensions; however, we are no longer limited to two-dimensional models (Duong 2007; Keating and Cherry 2007; Toms et al 2015). Depending on the question at hand, higher dimensional UDs may be more appropriate. For example, the accuracy of home range and space use of aerial or diving animals can be greatly improved by incorporating height or depth, respectively, as a third dimension in UD models (Zhu and Weng 2007; Cooper et al. 2014). For instance, Simpfendorfer et al. (2012) found that the overlap of space use among European eels (*Anguilla anguilla)* was significantly greater in two-dimensional (2D) home range models

than three dimensional (3D) models that included a depth dimension— likely because individuals may occupy the same horizontal location, but at different depths. Similarly, animals could alter their space use over time for various reasons such as tracking resources (Cooper et al. 2014). Conceivably, three-dimensional UD models would greatly improve the accuracy of space use in such cases, but including time as a third-dimension has rarely been used (Laver and Kelly 2008). Considering how space use varies in time may also resolve home range characteristics like size and shape, and may show how transitory resources within the environment influence an individual's space use (Keating and Cherry 2009, Clement et al. 2010). Here, I apply three dimensional (3D) UDs using spatial and temporal data to describe the space use over time of brood parasitic brown-headed cowbirds (*Molothrus ater;* hereafter cowbirds) in relation to short-lived resources and neighbouring conspecifics.

Obligate avian brood parasites, including cowbirds and cuckoos, lay their eggs in the nests of other 'host' species who subsequently care for the parasitic young as their own (Davies 2000). There is a large literature on the impact brood parasites may have on their hosts and in understanding the coevolutionary processes involved in host-parasite interactions (e.g. Rothstein 1990). Less is known, however, as to how parasites find nests, optimize their searching behaviour, and defend breeding resources from conspecifics (Rothstein et al. 1984; Clotfelter 1998; Honza et al. 2002), largely due to logistical difficulties involved in monitoring cryptic females while they search for nests (Norman and Robertson 1975; Wiley 1988; Kattan 1997; Banks and Martin 2001). The brood parasite-host system provides an ideal model for evaluating the utility of a time-dimension in UDs due to the ephemeral nature of host nests. Host nests are the essential

reproductive resource for a female parasite, but can only be successfully parasitized during a brief window of time early in development (Stausberger 1998; Fiorini et al. 2009). Thus, a breeding range is punctuated over space and time with discrete and brief laying opportunities and the timing and proximity of a parasite's activity can be readily correlated with these opportunities.

Brown-headed cowbirds are among the best-studied obligate brood parasite (see Lowther 1993), but information on important reproductive behaviours such as how individuals prioritize their nest searching to efficiently find host nests is lacking. Cowbirds find host nests primarily by quietly observing host activity (Norman and Robertson 1975, Wiley 1988, Kattan 1997), or occasionally by systematic searching (Fiorini and Reboreda 2006)— a process that requires substantial time and effort. Regardless of whether a nest is actually found the behaviour of the host parents may offer easily recognizable clues as to the developmental stage of that nest (Banks and Martin 2001). A brood parasite could capitalize on this information, forming dynamic maps of potential laying opportunities, and allot their time accordingly by nest searching in areas that likely have an active nest. Female cowbirds appear particularly well suited to handle the spatial processing demands associated with a parasitic lifestyle. Female cowbirds have an enlarged hippocampus relative to males— the brain region involved with spatial information processing (Sherry et al. 1993, Reboreda et al. 1996) and perform significantly better than males in spatial memory tasks (Guigueno et al. 2014). At present, little information is available on how the developmental stage of host nests may influence cowbird nest searching effort in the wild. Even more generally, habitat and resource

selection studies have not previously applied UDs that included a time dimension in this way to test whether relative activity varies with the availability of short-lived resources.

Territories can generally be defined as relatively exclusive areas that are actively defended against intruders (Davies and Houston 1984) and have been the subject of several studies on brood parasites (e.g. Clotfelter 1998; Martinez et al. 1998). Field studies using genetic parentage analysis point to the realized fecundity of cowbirds being relatively low (Hahn et al. 1999; Alderson et al. 1999; Strausberger and Ashley 2003; Woolfenden et al. 2003) compared with earlier studies that predicted high egg laying potential (Payne 1976; Scott and Ankney 1980, 1983; Jackson and Roby 1992; Kattan 1993). Consequently, the value of individual host nests is higher than previously accepted suggesting that individual cowbirds may benefit by monopolizing and defending laying opportunities from conspecifics. Nevertheless, evidence of cowbirds maintaining exclusive breeding territories is equivocal. Some studies strongly suggest that females defend breeding territories (e.g. Rothstein et al. 1984) but others show little evidence of territoriality (e.g. Fleshier 1985). I suspect that it is difficult to evaluate territoriality in brood parasites for two key reasons. First, unlike other birds, brood parasites are not obligated to return to a fixed nest site and are therefore free to shift their space use throughout the season— allowing for dynamic boundaries. In such cases, overlap among breeding ranges may be grossly overestimated if time is not accounted for. Second, because brood parasites are uniquely free of parental responsibilities, breeding (i.e. nest searching and laying), socializing, and feeding activities can be separated spatially and temporally (Thompson 1994). It is well documented that cowbirds tend to lay eggs just after dawn, search for nests throughout the morning, and commute to feeding areas for

the rest of the day (Rothstein et al. 1984; Thompson 1994; Gates and Evans 1998). Thus, active defense of a breeding territory may only be necessary early in the day when females are engaged with finding and parasitizing host nests. Hence, accounting for long-term variation in space use coupled with short-term variation in behaviour may be necessary to properly understand the territoriality of a brood parasite.

In this study, I use radio-telemetry data to describe the activity and behaviour of individual female brown-headed cowbirds within their breeding range and highlight the importance of considering time in both resource selection and territoriality studies. I applied recent product kernel methods (Duong 2007, Keating and Cherry 2009) that allow a time dimension to be included in a utilization distribution home range model. I then use these UDs to relate the relative probability of activity to the spatial and temporal availability of host nests. Specifically, I test whether cowbirds were more likely to be found around active versus inactive nests and test whether cowbird activity differs depending on the developmental stage of the nest in an area. A positive relationship between probability of use and nest availability would suggest that cowbirds monitor hosts and optimize their space use accordingly to efficiently search for nests. I also utilize multidimensional UDs to address possible territoriality in cowbirds. I compare the overlap among strictly spatial 2D breeding range estimates to that of 3D estimates that include a time dimension to: (1) quantify the degree of exclusivity among individual breeding ranges and; (2) identify limitations or biases of breeding range estimates that do not account for time. Further, I expected that on a short time scale cowbirds will become less territorial later in the day after laying and nest searching. To this end, I use behavioural observations of radio-tracked females to test whether group size and

proportion of aggressive interactions changed as the day progressed. Lastly, I use highly variable microsatellite DNA markers (Alderson et al. 1999, OT Longmire et al. 2001) to assign maternity to several cowbird eggs found in my study site in order to map laying ranges and visually inspect laying range exclusivity.

4.2 Methods

4.2.1 Study site

I monitored the movements and parasitism of radio-tagged female brown-headed cowbirds during two breeding seasons (2009-2010) from mid-May to late-July at Rithet's Bog— a 42 hectare nature sanctuary near Victoria B.C., Canada (4826 N, 12320 W). Cowbird parasitism has been monitored in detail at this site since 2000 on a population of resident song sparrow (*Melospiza melodia*) hosts. Other common hosts in the area include common yellow throats (*Geothlypis trichas*), yellow warblers (*Setophaga petechia*), and red-winged black birds (*Agelaius phoeniceus*), however, cowbird activity was only monitored at song sparrow nests. From 2000-2010 parasitism rates on song sparrows were high, ranging from 17 – 78% (48.3 ± 5.3%, mean ± SE; Swan et al. 2015). Migratory cowbirds arrive to the area in mid April and the first cowbird egg has typically been found at the study site a few weeks later (mean date: 30 April; range: 15 April – 16 May). The final cowbird egg of the season was found mid July (mean date: 7 July; range: 2 July – 14 July). Additional details regarding the study site can be found elsewhere (Zanette et al. 2003; 2006).

In 2009 and 2010 I searched the study site daily to locate song sparrow host nests. Once found, nests were monitored regularly (every one to three days) and during each visit to a nest a researcher recorded the number of host and cowbird eggs present. When present, cowbird eggs were removed from nests and replaced with model eggs to maintain clutch size and reduce the chance of nest abandonment. Cowbird eggs were then placed in an incubator until there was sufficient tissue development for genetic extraction (see below). I determined the age of a host clutch by candling eggs or by back-dating from hatch day.

4.2.2 Capture and radio-tracking

Cowbirds were captured using seven semi-permanent house traps (1.6m x 1.6m x 2m) positioned throughout the study site where trapping had previously proven successful. House traps were supplied *ad libitum* with water and bird seed and contained two female and three male live cowbird decoys. Decoy birds were captured from other sites at least two km away. On first capture, all cowbirds were fitted with a metal leg band for permanent identification and a unique combination of coloured leg bands for identification at a distance. A blood sample was also collected from the brachial vein ($\approx$50 μl) for parentage analyses (see below).

Eleven female cowbirds (five in 2009, six in 2010) were equipped with radio transmitters (1.8g, Holohil Systems, Carp, Ontario) and tracked throughout the season. These transmitters constituted less than 5% of an individual's body mass (36.4 ± 0.8 g, mean ± SE) and were attached using a figure-8 leg harness (Rappole and Tipton 1991). It is generally accepted that tracking technology less than 5% of body mass does not

substantially affect the behaviour or survival of flying animals (Aldridge and Brigham 1988). Transmitters were only given to females that were captured or observed in the study area for longer than five days to ensure that they were resident to the area and not migrating through (Wolfenden et al. 2001). In addition to the 11 radio-tracked birds, two cowbirds known to be resident based on my criteria were excluded from analyses because one was found dead, likely due to a cat based on the remains, and the other did not respond well to the transmitter when I tested her before release and hence, I removed the transmitter. Based on continual trapping and observations throughout the breeding season, I estimated that we caught all resident cowbirds in the study area.

I located birds using the homing method or via triangulation with a handheld receiver and Yagi antenna. To reduce error associated with triangulation, I confirmed that an individual was within 30 m prior to taking a compass bearing by removing the antennae from the receiver and listening for a signal. Systematic testing previously showed that a signal could be detected by a receiver after the antennae was detached when a transmitter is closer than 30 m. Location data were collected using "burst sampling"— a technique that allows many observations to be taken over short, regular time intervals (Barg et al. 2005). Bursts typically lasted one to two hours and successive observations within a burst were separated by short time intervals (10-20 minutes). Given that cowbirds are highly active and can easily move across their entire range in this amount of time (Swan personal observation) I felt that this method of sampling maximized the number of relocation points and provided biologically independent locations (Lair 1987, Barg et al. 2005). In total I obtained between 102 and 273 (160 ± 13, mean ± SE) relocation points per individual for the 11 radio-tracked birds— an

amount more than adequate to reliably create and analyze two-dimensional and three-dimensional utilization distributions (Seaman et al. 1994, Cooper et al. 2014).

Because of the cryptic nature of female cowbirds and the dense vegetation throughout much of my study site it was not always possible to visually confirm an individual's location. When possible however, I recorded the number of male and female cowbirds present with a focal individual (17% of 1974 total relocation points). If other females were present I classified behaviour of the focal individual as aggressive or non-aggressive. I define an interaction as aggressive whenever a female performed a chatter call (a vocalization often considered antagonistic between females, but also attractive to males: Dufty 1982; Burnell and Rothstein 1994; Ortega 1998), a heads-up display (bill pointed vertically towards another cowbird: Dufty 1982; Teather & Robinson 1984; Burnell and Rothstein 1994) or chased or was chased by another cowbird (Teather & Robinson 1984). If none of these three behaviours were observed, I recorded the interaction as non-aggressive. I assumed that aggressive interactions among individuals within my study site reflected territorial behaviour.

4.2.3 Parentage analysis using microsatellite DNA loci

I genotyped the blood from cowbird females and tissue collected from cowbird eggs at seven hypervariable microsatellite loci (Dpu16, Mau20, Mau25, Mau29 from Alderson et al. 1999; CB1, CB12, CB15 from Longmire et al. 2001). One primer of each locus was dye-labelled and microsatellites were amplified using polymerase chain reaction (PCR). Each PCR was conducted in a total volume of 10 μl and included the following: 10 mM of Tris-HCl, 50 mM of KCl, 0.1% Triton X-100, 0.2 mg/ml of BSA, 2.5 mM of $MgCl_2$,

0.2 mM of each dNTP, 0.1 - 0.4 mM of each primer, 0.5 U of Taq polymerase (Fisher Scientific GoTaq) and 20 - 100 ng of genomic DNA. Cycling conditions included an initial step of 180 seconds at 94ºC, followed by 29 cycles of 30 seconds at 94ºC, 90 seconds at the annealing temperature and 60 seconds at 72ºC, ending with a final step of 270 seconds at 72ºC. The PCR products were analyzed on an Applied Biosystems 3130 Genetic Analyzer according to the manufacturer's protocols, and alleles scored manually. I determined the identity of the genetic mother by comparing all offspring-adult female allele combinations at each locus. Adult females were excluded as a possible mother whenever they could not have contributed at least one of the alleles present in an offspring at each locus. I excluded the microsatellite loci Mau29 (Alderson et al. 1999) from this analysis because it failed to produce a sufficient number of peaks to be informative. I included both resident birds and migrating birds as potential maternal candidates. I did this to be more conservative when assigning maternity and to validate my trapping estimates of the number of breeding resident females in the area.

4.2.4 Creating Utilization Distributions

Two of my primary goals were to create utilization distributions to evaluate how the arrangement of host nests in space and time correlate with the spatial distribution of female cowbirds and to estimate the amount of overlap among females within their breeding range in space and time. A utilization distribution provides a continuous and probabilistic estimate of space use throughout an individual's range based on telemetry location density (Worton 1989; Millspaugh et al. 2006). Using a distribution of relative use instead of discrete location data in space use reduces error from telemetry accuracy

and allows for predictions in locations where animals were never actually observed because of discontinues monitoring (Kernohan et al. 2001; Moser and Garton 2007). Studies often use UDs to link probability of use with some habitat variable(s) given that the height of a UD represents the relative probability of an individual being at any given location (Marzluff et al. 2004; Millspaugh et al 2006). Others estimate spatial overlap among individuals by overlaying multiple distributions (e.g. Simpfendorfer et al. 2012; Cooper et al. 2014). Currently, however, there are almost no other practical ecological examples of UDs, which include a time dimension, being used for such purposes (but see Calenge et al. 2010).

4.2.5 Estimating relative activity around nests over time

To estimate how individual space use changed throughout the season I created utilization distributions for all radio-tracked birds that allowed smoothing of a distribution of relocation points in both space and time (Keating and Cherry 2009). I used a robust multi-dimensional product kernel method described in the adehabitat package for the R statistical program (R development team 2008) specifically designed to analyze UDs in space and time (Calenge 2006). This package returns a series of 2D UDs at set time periods (i.e. daily) thereby allowing me to estimate how relative spatial activity changed day to day (see Figure 4.1). After initial exploration of the data, I choose smoothing bandwidths of 100 m and 72 hours for the spatial and time dimensions respectively. Because I was primarily interested in the spatial distribution of cowbirds that were engaged in breeding activities, I only included locations recorded before 13:00 when constructing these UDs. Several previous studies report that female cowbirds tend to

parasitize and search for host nests during the morning and early afternoon (Thompson 1998, Rothstein et al. 1984).

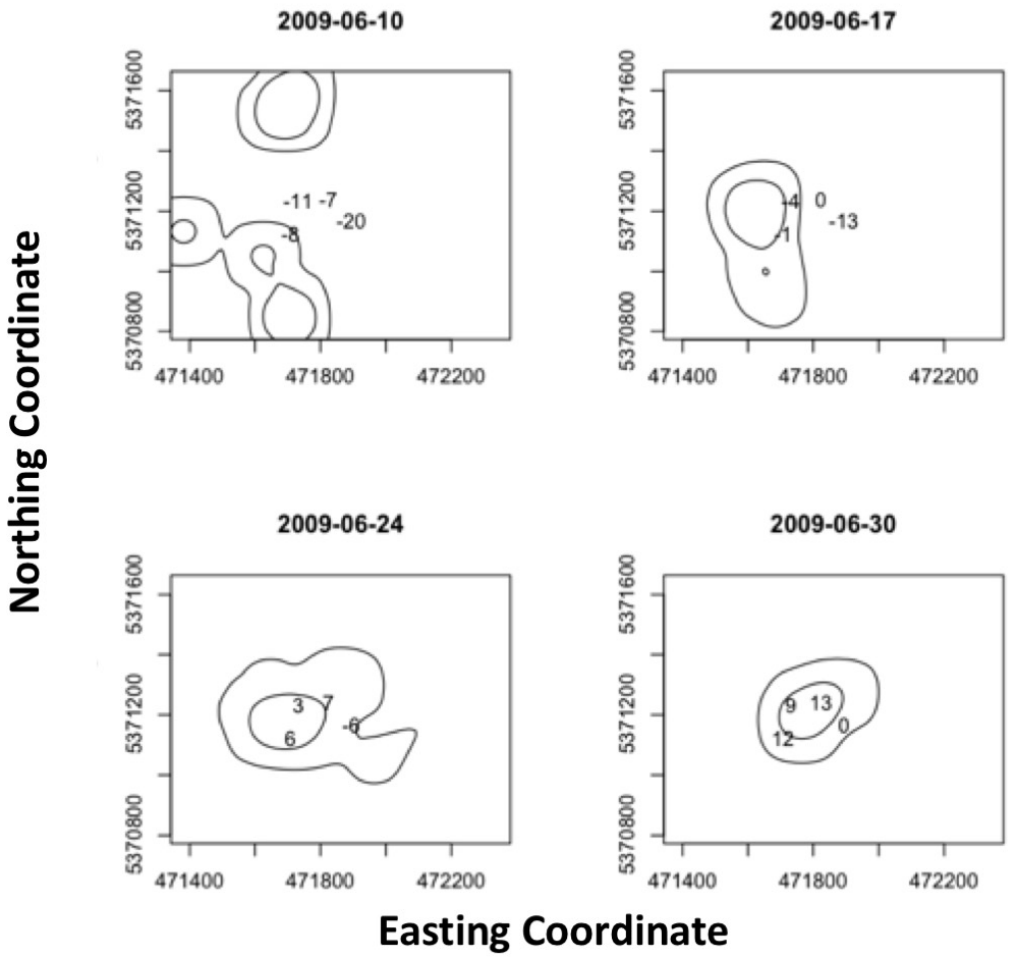

Figure 4.1: Cowbird home range and host nests visualized across time using adehabitat (Calenge 2006). The outer line in each panel represents the home range of a single bird estimated at different days. The numbers represent the locations of four song sparrow nests. The magnitude of the numbers indicate the age of the nest relative to the first day of incubation.

I assumed that the standardized height of a UD (hereafter "activity") reflects the overall effort a cowbird devoted to nest searching in the immediate area. This approach allowed me to quantify the activity of individual cowbirds around nests at different biologically meaningful stages of development (see below). For each cowbird, I quantified activity immediately around every nest (averaged over a 10m radius) that fell within the 95% isopleth of their estimated range for at least one day. A single measure of activity was recorded for each day around each nest that the bird was radio-tagged. I then assigned the activity estimates to biologically meaningful categories depending on the developmental stage of a nest on that day. These categories include: nest building; laying/early incubation (time from first egg to four days post-lay), late incubation (five days post-lay to hatch); brooding (chicks present); and inactive (no nesting activity). I averaged activity values within categories so that there was only one measure of activity for each bird-nest combination within each developmental category. Among these categories, laying and early incubation represents the suitable time period in which a cowbird egg will receive sufficient incubation to hatch (Swan et al. 2015, Fiorni and Reboreda 2006). The inactive period only included days when I was certain that no nest was active in that host's territory— typically three to seven days before or after. Obviously, any nest that were active outside of the period when cowbirds were radio-tracked could not be included in analyses.

4.2.6 Overlap among cowbird breeding ranges

I quantified spatial overlap among individual cowbirds using two-dimensional spatial UDs first, followed by three-dimensional UDs that included a time dimension, using the

“ks” package in the R statistical program (Duong 2007, see Figure 4.2). Unlike the method described above using the adehabitat package that estimates several daily two-dimensional UDs (Calenge 2006), this technique using the ks package allowed me to readily calculate a single measure of percent overlap among individual ranges and to compare the average amount of overlap between the 2D and 3D estimates. Because least-squares cross-validation (LSCV) repeatedly resulted in unrealistic under-smoothing, bandwidth selection was calculated using the plug-in method (Wand and Jones 2002, Gitzen et al. 2006, Duong 2007). UDs can be visualized at different isopleths, each of which describes equal probability of finding the focal bird within that boundary. Here I define an individual’s breeding range to be within the 95th isopleth (White and Garrott 1990, Laver and Kelly 2008, Ehrenberg and Steig 2003, Zhu and Weng 2007, Simpfendorfer et al. 2012). I estimated 2D breeding ranges using spatial GPS data (UTMs) and 3D breeding ranges using the same spatial input plus the Julian date on which the location was recorded. As above, I only used locations recorded before 13:00 when constructing UDs for each individual.

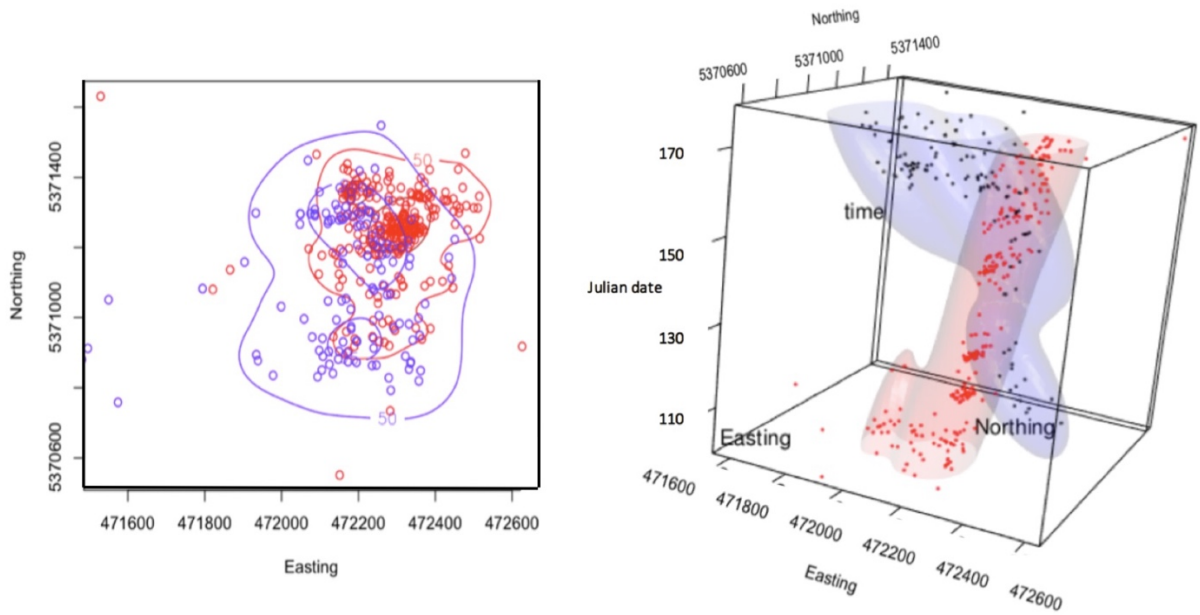

Figure 4.2: Overlapping 95% utilization distributions of two neighbouring brown-headed cowbird females in two-spatial dimensions (left panel) and three space and time dimensions (right panel). Easting and northing are UTM coordinates in meters.

I calculated the percent overlap among individual ranges from each model following Simpfendorfer et al. (2012). I then calculated the amount of overlap among birds in a pairwise approach wherein I determined the overlap of each individual with every other individual. Overlap was calculated for both the area of two-dimensional spatial UDs and the volume of three-dimensional space-time UDs. I changed the amount of overlap to a proportion by dividing the area/volume of the overlap by the total area/volume for an individual. For a given pair of birds, I calculated overlap only using relocation points that were collected when both birds were equipped with radio-transmitters. For example, if one bird was tracked from 1 to 30 June and another was tracked from 5 June to 3 July, overlap between these individuals was calculated using points collected between 5 and 30 June. This ensured that any lack of overlap was genuine and not simply the result of different sampling periods.

4.2.7 Statistical analyses

Data from individual cowbirds were included in analyses in all cases whenever more than 80 relocation points were collected— the minimum sample size required for 3D UD modelling (Cooper et al 2014). I hypothesized that cowbirds would prioritize their nest searching efforts in areas that contained active nests or nests at a suitable stage for parasitism. I performed a linear mixed model to test for differences in activity among stages of nest development (Building, Early Incubation, Late Incubation, Brooding, and Non-Active). Standardized activity was the response variable and nest stage was the fixed explanatory variable. Individual nest and cowbird identity were included as random explanatory variables. If cowbirds have dynamic territorial boundaries throughout the

season one would expect a fundamental bias in the amount of home range overlap using 2D spatial estimates. Such bias would manifest as more overlap among 2D vs 3D estimates because individuals may occupy the same location, but at different times. I tested for such a possibility using an ANOVA, with proportion of overlap as response variable and UD type (2D or 3D) as the explanatory variable. I included cowbird identity as a within-subjects factor. Proportions were arcsine-transformed prior to analysis. To test my hypothesis that female cowbirds become less territorial as the day progresses we tested for a correlation between the time of day that a cowbird was observed and the number of other cowbirds in the immediate area. I also tested for a correlation between time of day and the proportion of aggressive interactions among individuals. I averaged the number of male and female cowbirds observed with each focal bird over 3-hour blocks of time throughout the day (i.e. before 6:00, 6:01-9:00, … , 18:01-21:00) . I then calculated the Spearman rank correlation between group size and time period. Similarly, for each bird, I calculated the proportion of aggressive vs non-aggressive interactions with other females during each time period. I then performed a logistic regression to test for a correlation between this proportion and time period.

4.3 Results

4.3.1 Relative activity around nests over time

Consistent with my prediction that female cowbirds prioritize their nest searching in areas that were likely to contain a viable nest, I found a significant difference in cowbird

activity (standardized UD heights) around nest sites among the different stages of development (No nest, Building, Laying/Early incubation, Late incubation, Brooding; $X^2_{(4)} = 16.6$; P = 0.002; Fig 4.3). Post-hoc Tukey's tests among the nest stages revealed that there was no significant difference in activity among any of the developmental categories where a nest was actually present in an area (i.e. build, early inc, late inc and brood stages), but all four of these stages showed significantly more activity that when there was no active nest in the area.

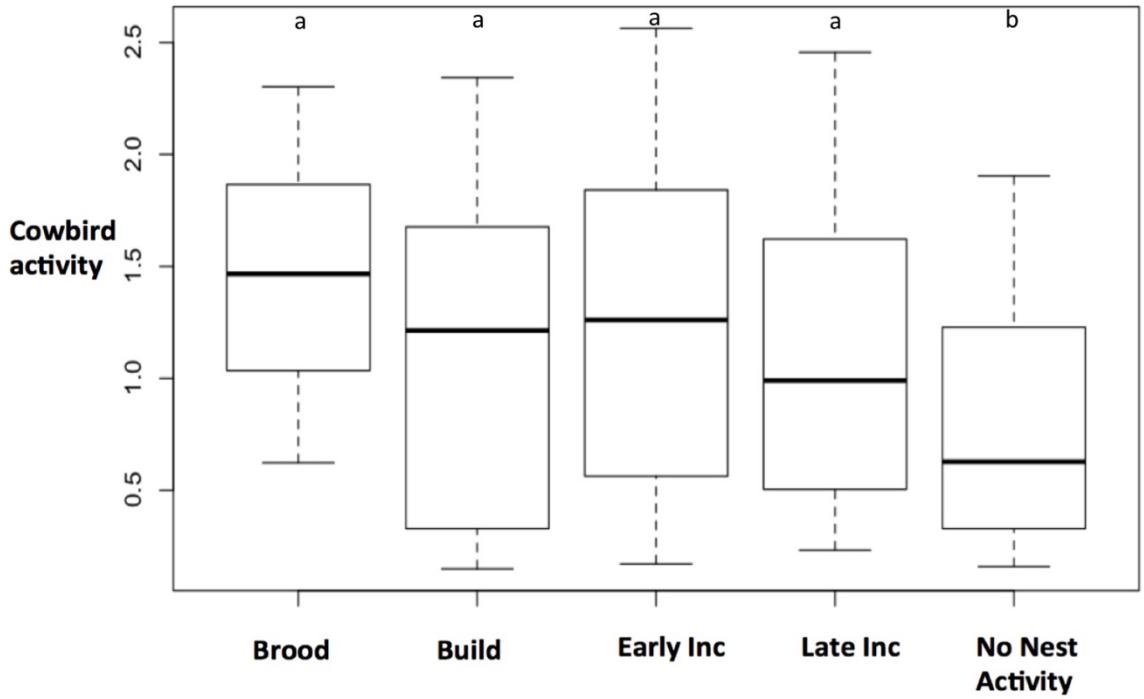

Figure 4.3: Relative activity of cowbird females (standardized height of utilization distributions) around nests across different stages of development.

4.3.2 Overlap among cowbird breeding ranges: two-versus three-dimensions

I quantified breeding range size and overlap among breeding ranges for all possible pairs (five in 2009, six in 2010) of cowbird females using 2D spatial and 3D spatio-temporal UDs. For comparison with other studies, the mean size of female breeding ranges over the entire season based on my 2D estimates was 27.5 ± 3.6 ha (mean ± SE). As expected if cowbirds have dynamic breeding range boundaries throughout the season, my results indicate that the 2D models overestimate the amount of overlap among neighbouring ranges by nearly double (20%). Almost universally, 3D estimates of overlap were smaller than their corresponding 2D estimates (Figure 4.4). Overall the average overlap among birds using 2D estimates (41.4 + 3.2%) was significant greater that using 3D estimates (21.1 + 2.2%; $F_{1,10} = 64.01$; $P < 0.001$); however, I found a fairly wide range overlap among individual breeding ranges calculated using both 2D (range = 0-95%) and 3D UDs (range = 0-57%).

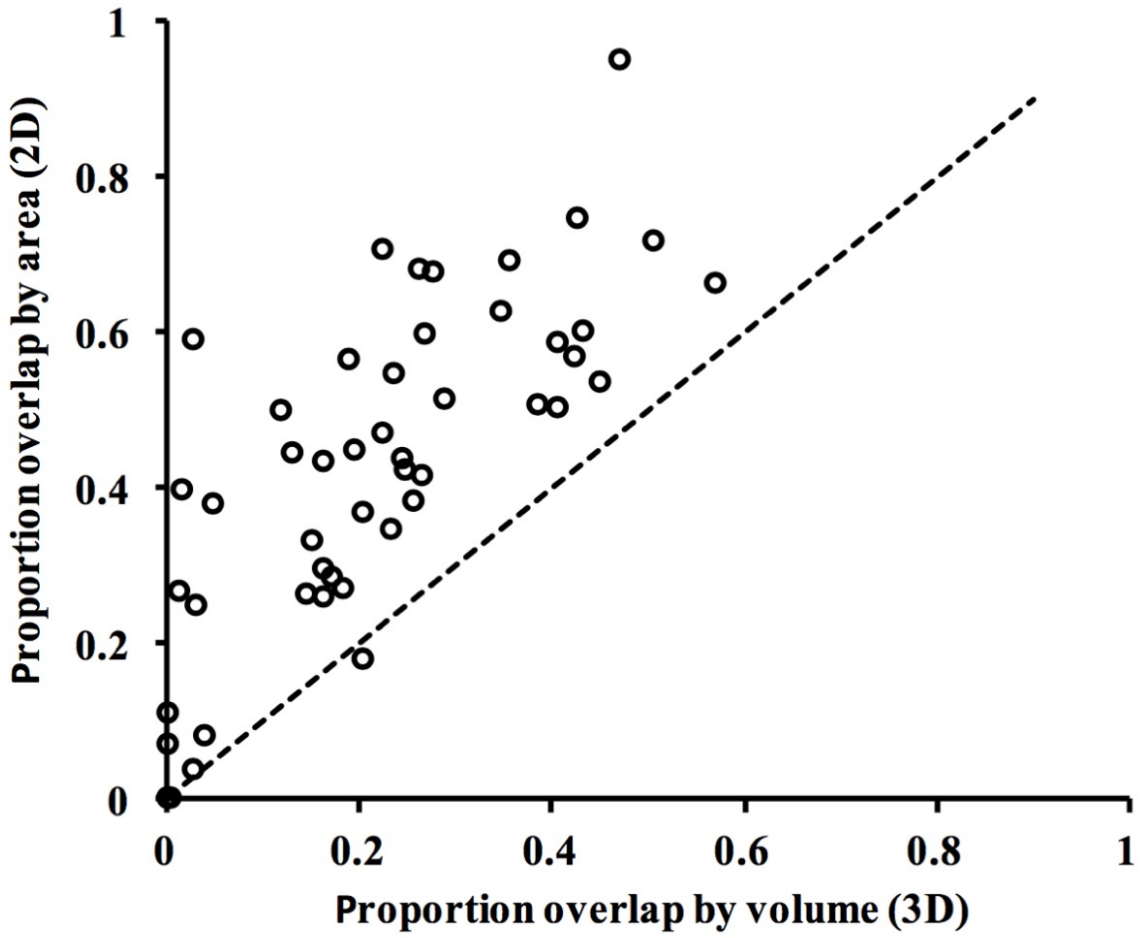

Figure 4.4: Comparison of two-dimensional spatial and three-dimensional spatio-temporal estimates of home range overlap among female cowbirds. Points above the line indicate overestimation of overlap using two-dimensional spatial methods.

4.3.3 Daily patterns in group size and aggression

Female cowbirds tended to follow a similar pattern of behaviour throughout the day, which is constant with my prediction that territoriality should abate as the day progresses and bolsters my claim that breeding territories should be estimated using relocation points collected before 13:00 (see above). Individuals were usually solitary in the morning and early afternoon and later congregated in groups. Typically, before 13:00, focal individuals were observed with fewer that one female or male in the immediate area and could be found in significantly larger groups later in the afternoon and evening (Figure 4.5; Spearman: number males, $t = 7.7$, $R = 0.38$, $P < 0.001$; number females, $t= 8.2$, $R = 0.40$, $P= < 0.001$). Furthermore, when another female was observed with a radio tagged bird it was much more likely that the interaction would be aggressive early in the day, but not so later on. Overall, the proportion of aggressive interactions among females significantly decreased as the day progressed (Logistic regression, $P = 0.002$, Figure 4.6). Indeed, over 70% of interactions among females before 13:00 could be categorized as aggressive, whereas fewer than 15% of interactions were considered aggressive after 15:00 (Figure 4.6). This apparent drop in aggression and increase in group size as the day progressed typically coincided with individuals traveling away from host abundant areas to locations with large trees, bird feeders and/or community gardens.

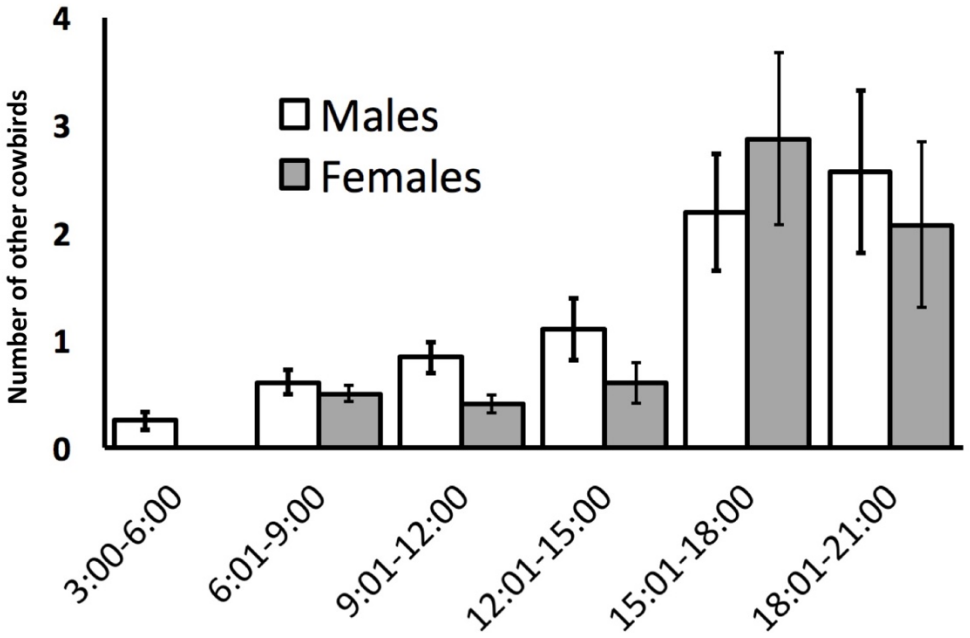

Figure 4.5: Number of male and female cowbirds (mean ± SE) observed with radio-tracked females throughout the day.

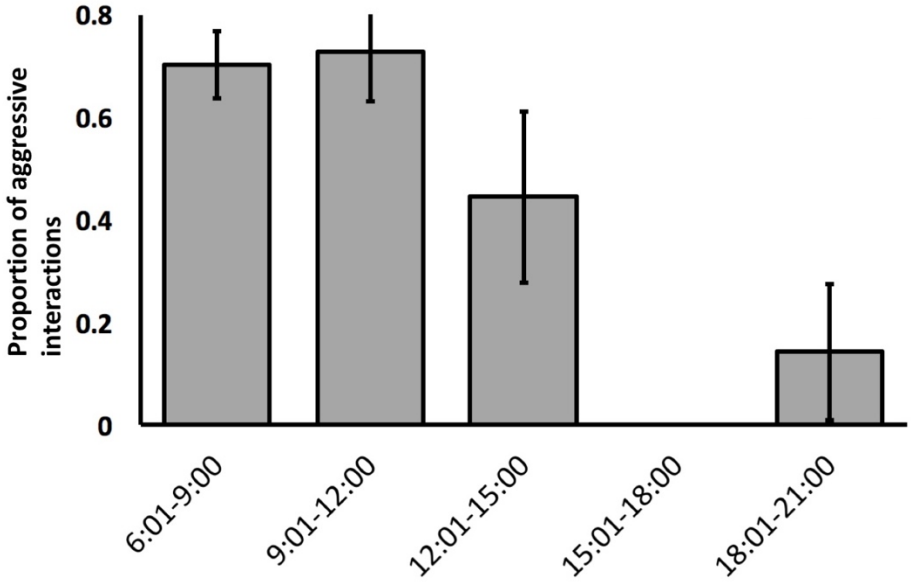

Figure 4.6: Proportion of aggressive interactions among female cowbirds throughout the day.

4.3.4 Egg-laying areas

Figure 4.7 shows the two-dimensional home ranges (95% UD isopleth)and location of parasitized nests for cowbirds in 2009. The location of parasitized nests suggests that female cowbirds generally have nearly exclusive egg laying ranges that are in close proximity to one another. I estimated egg laying areas for individual females as the minimum convex polygon surrounding the location of nests they had parasitized (as per Alderson et al. 1999). This was done for females that laid eggs in at least three different nests as inferred from genetic analyses (N = 4, Figure 4.7). The size of these areas ranged from 1.6 to 6.2 ha (mean ± SE = 3.1 ± 2.2). I was not able to calculate the egg-laying areas in three cases where females laid in fewer than three nests. Egg laying overlap was generally small, however, nests parasitized by different females could be as close as 17 m. I found three cases where a nest was multiply parasitized by different females.

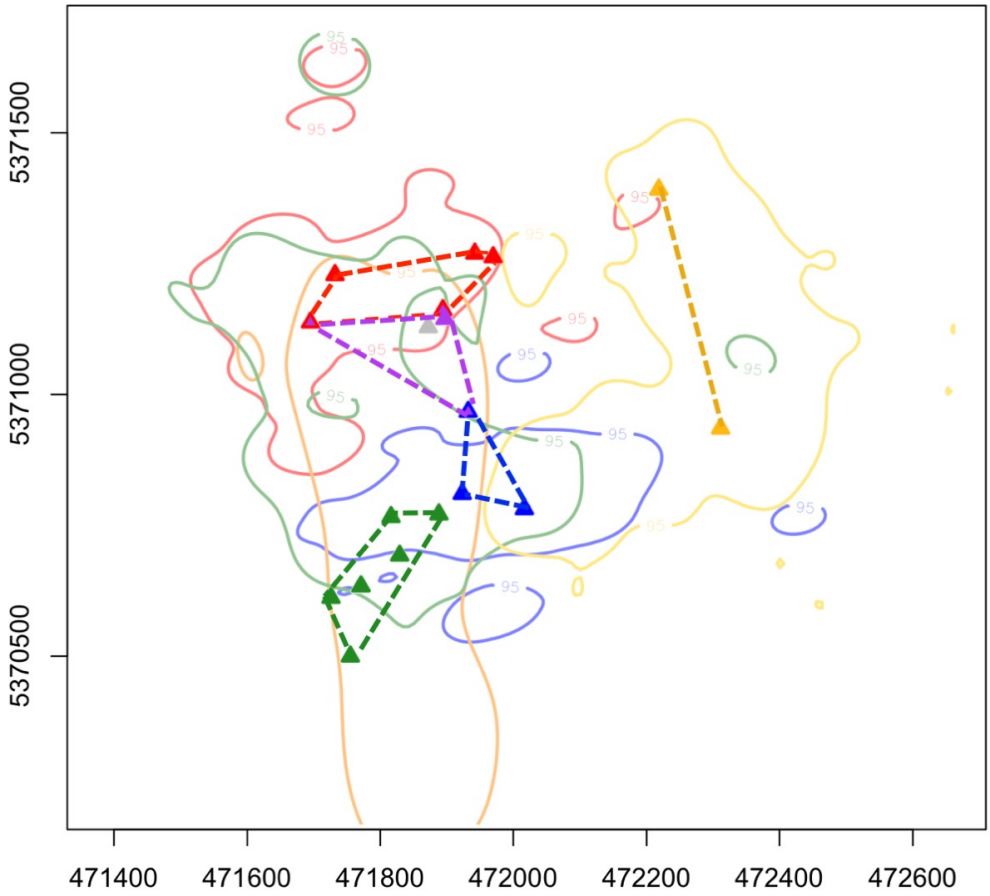

Figure 4.7: Home ranges and locations of host nests parasitized by female cowbirds in 2009. Each colour represents a different cowbird. Solid lines represent home range boundaries defined by the 95% isopleth of two dimensional utilization distributions. Triangles represent the location of specific parasitized nests. Triangles with multiple colours indicate multiple parasitism. Egg laying areas for females laying eggs in at least three different nests are shown as minimum convex polygons (dashed lines) encompassing the locations of parasitized nests. Single dashed lines connect two nests parasitized by the same female. Easting and northing are UTM coordinates in meters.

4.4 Discussion

My results clearly demonstrate that a time dimension can be an important consideration in resource selection and home range studies and can lead to important ecological revelations. This is especially true in cases where probability of occurrence varies over time according to the availability of some short-lived resource(s) or when space-use boundaries drift over time. Here, I am among the first to demonstrate the practical application of utilization distributions that include a time dimension (Calenge 2010). I found that the relative likelihood of finding a female cowbird at some point within their breeding range is correlated with the availability of a host nest in the immediate area. These results suggest that rather than methodical searching over their entire breeding range (Fiorini and Reboreda 2006), female cowbirds concentrate their nest searching efforts in areas where they are more likely to find a nest. Evidence of this kind of sophisticated searching optimization may go undiscovered using strictly spatial analyses. Accounting for seasonal variation in space use also allowed for a more realistic understanding of the amount of overlap among individuals within their breeding ranges. I found that 2D home range estimates overestimated the amount of home range overlap by nearly double when compared to 3D spatio-temporal estimates. Likely, individuals occurred at similar locations, but at different times throughout the season. In addition to seasonal variation, I also found evidence that territorial behaviour seems to diminish throughout the day likely because cowbirds separate breeding and non-breeding behaviours spatially and temporally. Cowbird group size increased and aggressive interactions decreased significantly as the day progressed.

The relative probability of a cowbird occurring in an area was significantly greater when there was an active nest present. These results are entirely in line with my prediction that cowbirds attend to host activity and prioritize their space use in areas that contain a nest (Honza et al. 2002). It is generally thought that cowbirds primarily find nests by monitoring host behaviour and that the process takes a substantial amount of time (e.g. Banks and Martin 2001). Female cowbirds have the potential to lay large quantities of eggs throughout the breeding season and their fecundity is almost certainly limited by the number of suitable host nests they can find and exploit (Payne 1977, Wyllie 1981, Scott and Ankney 1983). By attending to host behaviour and adjusting their space use accordingly a cowbird could greatly increase nest searching efficiency. Although I did not measure host activity directly, it seems the likely driver of my results for several reasons. First, multiple species of brood parasite have been observed monitoring host individuals for extended periods (e.g. Honza et al. 2001). Second, the overall activity of hosts around their nests during nest building can affect the probability of parasitism (Gill et al. 1997; Banks and Martin 2001). Third, several hosts including song sparrows exhibit distinct behavioural cues depending on the developmental stage of a nest or whether they have an active nest at all (Swan personal observation). Female cowbirds appear to have relatively advanced spatial processing ability (Sherry et al. 1992; Reboreda et al. 1996; Guigueno et al. 2014) and it would not be surprising if their spatial cognition was uniquely adapted to form dynamic maps of where and when host nests are available within their breeding range. Note that my results do not definitively show that higher activity around a nest necessarily results in parasitism. Indeed, I did not monitor all host nests in my study site and many nests I did were never parasitized. I was,

however, able to demonstrate that the existence of a nest influences the likelihood of a cowbird being present in space and time.

Contrary to my expectations, I did not find any difference in the probability of occurrence around nests at different stages of development. Cowbirds and cuckoos certainly tend to disproportionately parasitize nests early in development, so why do we not find a difference in probability of occurrence? Possibly, cowbirds do not infer the developmental stage of a nest from host behaviour alone and the decision to parasitize may occur only after a nest is discovered. Indeed, there is strong evidence that cowbirds determine the suitability of a nest for parasitism from the nest contents (White et al. 2007, 2009, Swan et al. 2015)— requiring close inspection. Alternatively, my presupposition that cowbirds should attempt to only find early stage nests may be wrong. Brood parasites often visit nests without laying perhaps to check on their status (e.g. Scott 1992; Honza et al. 2002). Common cuckoos, for example, have been shown to significantly increase their visits to an area once a nest appeared, but show no difference in time around nests whether they lay or not (Honza et al. 2002). There is also mounting evidence that cowbirds act as nest predators destroying nests discovered too late in the nesting cycle to be suitable for parasitism, thereby creating future opportunities to parasitize the host's resetting attempts (Farming hypothesis: Arcese et al. 1992, 1996). Searching for late-stage nests may be well worth the effort especially when predatory cowbirds are able to learn the approximate timing and location of the subsequent resetting attempt (Arcese et al. 1996, Hoover and Robinson 2007). In order to tease apart why cowbirds are equally likely to be found around early and late-stage nests, future studies may need to monitor behaviour directly.

My two dimensional spatial estimates of UDs indicate a high degree of overlap among individual cowbird breeding ranges. These results are similar to those found in other studies (e.g. Teather and Robinson 1985) and could lead to the conclusion that females share large areas where they compete for every laying opportunity. Three dimensional estimates of UDs that include a time dimension, however, show that cowbird ranges may overlap spatially, but often do so at different times throughout the season. As a result, the amount of overlap from the 3D estimates was nearly half that of the 2D estimates. This can lead to the drastically different conclusion that cowbirds in my study site may segregate themselves, thereby reducing competition for individual nests (Langmore et al. 2007). These results alone do not indicate that the cowbirds in my study maintain completely exclusive breeding ranges. Indeed, the 3D space-time UDs occasionally show fairly large amounts of overlap among neighbours; although, visual inspection of where particular females lay seems to indicate fairly separate laying ranges (Figure 4.7). I do, however, suggest a systematic bias when studying cowbird spatial use without considering time. While I corroborate other findings that cowbirds hold relatively constant breeding ranges (Teather and Robinson 1985, Scardamaglia and Reboreda 2014), it seems that the boundaries shift subtly over time. Future consideration of why cowbirds shift their space use can only increase our understanding of competition for nests among neighbours.

Understanding how spatial activity varies with time may be critical when studying territorial behaviour among neighbouring cowbirds. This is true on both a seasonal and daily time scale. Female cowbirds tend to be asocial in the morning while on their breeding ranges and congregate with others later in the afternoon while feeding

(Rothstein et al. 1984). Accordingly, my results corroborate other studies that report the mean number of cowbirds in a group increasing throughout the day. (Thompson 1994; Gates and Evans 1998; Goguen and Mathews 2001) and further show that females had proportionally fewer aggressive interactions as the day progressed. So, females appear to spatially segregate themselves while engaged in breeding activities and also actively defend breeding areas to some degree. The breakdown of this apparent territorial behaviour later in the day likely reflects the spatial and temporal separation of breeding, feeding and socializing behaviour commonly and uniquely observed in cowbirds (Dufty 1982; Rothstein 1984, 1986; Thompson 1994). Flocking together later in the day may be important for social reasons (White et al. 2007), predator detection (Lima 1993), or may simply result from birds congregating at prime feeding locations (Rothstein et al. 1986), however, the actual advantages remain largely speculative. Detailed analyses of the benefits from following this pattern of being solitary on breeding ranges and highly social later in the same day would be an informative topic of future study.

The resolution of the utilization distributions in space and time in this study is admittedly crude. Typically, a single researcher was recording locations of a single cowbird, leaving gaps in my records of where every individual was at any given time. That said, I want to emphasize the novel results I was able to generate despite these sampling limitations. With higher resolution, even more meaningful questions could be asked. For example, do cowbirds spend more time around nests they are about to parasitize or is equal effort given to all nests in their breeding range? Do females defend individual host nests or do they patrol and defend breeding range boundaries (Martinez et al. 1998). Do they spend more time after they parasitize to either defend against other

cowbirds or to ensure that the host has not rejected the egg (Hoover and Robinson 2007)? Do they return to an area after the have destroyed a nest to find the new resetting attempt (as per the farming hypothesis; Arcese et al. 1996). For this current study I simply lacked the power to test for such possibilities, however, as equipment becomes increasingly miniaturized and monitoring becomes better automated, answers to these questions and more may be answered.

Incorporating, multiple dimensions into animal movement and homerange studies may provide much more accurate and realistic estimates of how animals occupy space (Cooper et al. 2014). Time has by in large been ignored when creating utilization distributions (Laver and Kelly 2008) and, to my knowledge, this is the first study to relate a 3D probabilistic measure of space use over time to resource availability or to examine territory characteristics. Needless to say, past studies recognize that animal space use can change over time, however, these typically analyze strictly-spatial UDs at discrete time periods instead of incorporating time into a continuous model (E.g. Ehrenberg and Steig 2003; Simpfendorfer et al. 2012; Cooper et al. 2014). Although I present one example here, the application of multidimensional space-time UDs is clearly relevant to a wide range of ecological questions (Keating and Cherry 2009) and should be considered in future research.

4.5 References

Adams ES. 2001. Approaches to the study of territory size and shape. Adv Stud Behav. 32:277-303.

Alderson GW, Gibbs HL and Sealy SG. 1999. Determining the reproductive behaviour of individual brown-headed cowbirds using microsatellite DNA markers. Anim Behav 58:895-905.

Aldridge HDJN and Brigham RM. 1988. Load carrying and maneuverability in an insectivorous bat: A test of the 5% "rule" of radio-telemetry. J. of Mammal. 69:379-382.

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. Proc Natl Acad Sci. U.S.A. 93:4608-4611.

Barg JJ, Jones J and Robertson RJ. 2005. Describing breeding territories of migratory passerines: Suggestions for sampling, choice of estimator, and delineation of core areas. J. Anim. Ecol. 74:139-149.

Belant JL, Millspaugh JJ, Martin JA and Gitzen RA. 2012. Multi-dimensional space use: the final frontier. Frontiers Ecol. and the Environ. 10:11-12.

Calenge C, Guillemain M, Gauthier-Clec M and Simon G. 2010. A new exploratory approach to the study of the patio-temporal distribution of ring recoveries: the example of Teal (*Anas crecca*) ringed in Camargue, Southern France. J. Ornithol. 151:945-950.

Calenge C. 2006. The package adehabitat for the R software: A tool for the analysis f space and habitat use by animals. Ecol. Modeling 197:516-519.

Cooper NW, Sherry TW, and Marra PP. 2014. Modeling three-dimensional space use and overlap in birds. Auk. 131:681-693.

Darley J.A. 1968. The social organization of breeding Brown-headed cowbirds. Unpubl. Ph.D. diss. University of Western Ontario, London.

Darley JA. 1982. Territoriality and mating behavior of the male brown-headed cowbird. Condor 84:15-21.

Darley JA. 1983. Territorial behaviour of the female brown-headed cowbird (*Molothrus after*). Can. J. Zool. 61:65-69.

Dufty AM. 1982a. Movements and activities of radio-tracked brown-headed cowbirds. Auk 99:316-327.

Dufty AM. 1982b Response of Brown-headed cowbirds to simulated conspecific intruders. Anim. Behav. 30:1043-1052.

Duong T. 2007. ks: Kernel density estimation and kernel discriminant analysis for multivariate data in R. J. Stat. Software 21:1-16.

Ehrenberg JE and Steig TW. 2003. Improved techniques for studying the temporal and spatial behavior of fish in a fixed location. J. Marine Sci. 60:700-706.

Fiorini VD and Reboreda JC. 2006. Cues used by shiny cowbirds (*Molothrus bonariensis*) to locate and parasitise chalk-browed mockingbird (*Mimus saturninus*) nests. Behav. Ecol. Sociobiol. 60:379-385.

Fleisher RC. 1985. A new technique to assess the dispersion of eggs of individual brood parasites. Behav. Ecol. Sociobiol. 17:91-99.

Gates EJ and Evans DR. 1998. Cowbirds breeding in the central Appalachians, p. spacial and temporal patterns and habit selection. Ecol. Applications 8:27-40.

Gitzen RA, Milllspaugh JJ and Kernohan BJ. 2006. Bandwidth selection for fixed-kernel analysis of animal utilization distributions. J. Wildlife Manage. 70:1334-1344.

Goguen CB, Curson DR and Mathews NE. 2011. Costs of multiple parasitism for an avian brood parasite, the Brown-headed cowbird (*Molothrus after*). Can. J. Zool. 89:1237-1248.

Gouge CB and Mathews NE. 2001. Brown-headed cowbird behaviour and movements in relation to livestock grazing. Ecol. Applications 11:1533-1544.

Gow EA, Done TW and Stutchbury BJM. 2011. Radio-tags have no behavioural or physiological effects on a migratory songbird during breeding and molt. J. Field Ornithol. 82:193-201.

Guigueno MF, Snow DA, MacDuogall-Shackleton SA and Sherry D. 2014. Female cowbirds have more accurate spatial memories than males. Biol. Letters

Honza M, Taborsky B, Teuschi Y, Vogl W, Moksnes A and Roskaft E. 2002. Behaviour of female Common Cuckoos, *Cumulus caners*, in the vicinity of host nests before and during egg laying: a radiotelemetry study. Anim. Behav. 64:861-868.

Kattan GH.1997. Shiny cowbirds follow the 'shotgun' strategy of brood parasitism. Anim. Behav. 53:647-654.

Keating KA and S Cherry S. 2009. Modeling utilization distributions in space and time. Ecology 90:1971-1980.

Kernohan BJ, Gitzen RA and Millspaugh JJ. 2001. Analysis of animal space use and movements. In Millspaugh JJ and Marzluff JM, editors. Radio tracking and animal populations. Academic, San Diego, California, USA. Pp 125-166.

Lair H. 1987. Estimating the location of the focal center in red squirrel home ranges. Ecology 68:1092-1101.

Laskey AR. 1950. Cowbird behavior. Wilson Bulletin. 62:157-174.

Laver PN and Kelly MJ. 2008. A critical review of home range studies. J. Wildlife Manage. 72:290-298.

Longmire JL, Roach JL, Maltbie M, White PS, Tatum OL, Makova KD, and Hahn DC. 2001. Tetranucleotide microsatellite markers for the brown-headed cowbird *Molothrus ater*. Journal of Avian Biology 32:76-78.

Marzluff JM, Millspaugh JJ, Hurvitz P and Handcock MS. 2004. Relating resources to a probabilistic measure of space use: forest fragments and Steller's jays. Ecology 85:1411-1427.

Marzluff JM, Millspaugh JJ, Hurvitz P and Handcock MS. 2004. Resource utilization of an avian nest predator: relating resources to a probabilistic measure of animal use. Ecology 85:1411-1427.

Mattison J, Persson J, Andrén and Segerstöm P. 2011. Temporal and spatial interactions between an obligate predator, the Eurasian lynx (*Lynx lynx*), and a facultative scavenger, the wolverine (*Gulo gulo*). Can. J. Zool. 89:79-89.

Millspaugh JJ, Brundige GC, Gitzen RA and Raedeke KJ. 2000. Elk and hunter space-use sharing in South Dakota. J. Wildlife Manage. 64:994-1003.

Millspaugh JJ, Nielson RM, McDonald L, Marzluff JM, Gitzen RA, Rittenhouse CD, Hubbard MW and Sheriff SL. 2006. Analysis of resource selection using utilization distributions. J. Wildlife Manage. 70:384-395.

Norman R and Robertson RJ. 1975. Nest searching behavior in the Brown-headed Cowbird. Auk 92:610-611.

R Development Core Team. 2009. R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org [accessed 1 June 2016]

Rappole JH and Tipton AR. 1991. New harness design for attachment of radio transmitters to small passerines. J. Field Ornithol. 62:335-337.

Reboreda JC, Clayton N and Kacelnik A. 1996. Species and sex differences in hippocampus size in parasitic and non-parasitic cowbirds. Neuroreport 7:505-508.

Robinson SK, Rothstein SI, Brittingham MC, Petit LJ and Gryzbowski JA. 1995. Ecology and behaviour of cowbirds and their impact on host populations. In Matin T and Finch D, editors. Ecology and management of Neotropical birds. Oxford University Press, Oxford, U.K. Pp 428-460.

Robinson SK, Thompson FR, III, Donovan TM, Whitehead DR and Faaborg J. 1995. Science 267:1987-1990.

Rothstein SI and Robinson SK. 1998. Parasitic birds and their hosts: studies in coevolution. Oxford, UK. Oxford University press.

Rothstein SI, Verner JA and Stevens E. 1984. Radio-tracking confirms a unique diurnal pattern of spatial occurrence in the Brown-headed Cowbird. Ecology 65:77-88.

Seaman DE, Millspaugh JJ, Kernohan BJ, Brundige GC, Raedeke KJ and Gitzen RA. 1999. Effects of sample size on kernel home range estimates. J. Wildlife Manage. 63:739-747.

Sherry D., Forbes M., Khurgel M. and Ivy G. 1992. Females have a larger hippocampus than males in the brood-parasitic Brown-headed Cowbird. Proc. Nat. Acad. Sci. USA 90:7839-7843.

Simpfendorfer CA, Olsen EM, Heupel MR and Moland E. 2012. Three-dimensional kernel utilization distributions improve estimates of space use in aquatic animals. Can J Fish Aquatic Sci 69:565-572.

Spottiswoode CN, Kilner RM and Davies NB. 2012. Brood parasitism. In. Royal N, Smiseth PT and Kolliker M, Editors. The Evolution of Parental Care. Oxford University Press, Oxford, UK. pp. 225-243.

Swan DC, Zanette LY and Clinchy M. 2015. Brood parasities manipulate their hostsg: experimental evidence for the farming hypothesis. Anim. Behav. 105:29-35.

Teather K and Robinson RJ. 1985. Female spacing patterns in the Brown-headed cowbird. Can. J. Zool. 63:218-222.

Thompson FR III. 1994. Temporal and spatial patterns of breeding Brown-headed Cowbirds in the midwestern United States. Auk 111:979-990.

Van Winkle W. 1975. Comparison of several probabilistic home-range models. J. Wildlife Manage. 39:118–123.

Wand MP and Jones MC. 1993. Comparison of smoothing parameterizations in bivariate kernel density estimation. J. Amer. Stat. Assoc. 88:520-528.

Wand MP and Jones MC. 2002. Kernel smoothing (Vol. 60). Chapman and Hall/CRC.

White DJ, Ho L and Freed-Brown G. 2009. Counting chicks before they hatch: Female cowbirds can time readiness of a host nest for parasitism. Psycol Sci. 20:1140-1145.

White DJ, Ho L, de los Santos G and Godoy I. 2007. An experimental test of preferences for nest contents in an obligate brood parasite, *Molothrus ater*. Behav Ecol. 18:922-928.

White GC and Garrott RA. 1990. Analysis of wildlife radio-tracking data. Academic Press, San Diego California, USA.

Wiley JW. 1988. Host selection by the shiny cowbird. Condor 90:289-303.

Woolfenden BE, Gibbs HL and Sealy SG. 2001. Demography of brown-headed cowbirds at Delta Marsh, Manitoba. Auk 118:156-166.

Worton BJ. 1987. A review of models of home range for animal movement. Ecol. Model. 38:277-298.

Worton BJ. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70:164-168.

Zanette L, Clinchy M and Smith JNM. 2006. Combined food and predator effects on songbird nest survival and annual reproductive success: results from a bi-factorial experiment. Oecologia 147:632-640.

Zanette L, Smith JNM, van Oort H and Clinchy M. 2003. Synergistic effects of food and predators on annual reproductive success in song sparrows. Proc. Roy. Soc. London B. 270:799-803.

Zhu L and Weng W 2007. Catadioptric stereo-vision system for the real-time monitoring of 3D behaviour in aquatic animals. Physiol Behave 91:106-119.

Chapter 5

5 General Discussion

5.1 Summary

Parasites exhibit a wide variety of strategies to maximize the successful transmission of themselves and/or their offspring, by exploiting hosts. One such strategy occurs when parasites manipulate host behaviour in a way that increases their probability of transmission to an uninfected host. In Chapters 2 and 3 of this thesis, I examined whether brood parasitic brown-headed cowbirds (*Molothrus ater*) attack and cause nest failure in late-stage, and hence, inappropriate host nests which theory suggests they may do to parasitize the replacement nests at an opportune time— effectively manipulating their host's reproductive behaviour and improving their own transmission (farming hypothesis). Consistent with the predictions of the farming hypothesis, I found evidence that cowbirds are substantial predators of the nests of their hosts. Furthermore, cowbirds appear attuned to the reproductive stage of their hosts and act accordingly by destroying non-parasitized clutches they find late in the nesting cycle. They likely determine the developmental stage of nest either directly by puncturing a portion of the clutch or indirectly by attending to the absolute number of eggs present. I also found that the re-nesting attempts of hosts following suspected cowbird attacks were parasitized at significantly higher rates than those following non-cowbird predators suggesting that cowbirds gain reproductive benefits from their predatory actions. My Monte Carlo simulations suggest that farming behaviour may yield increased reproductive output not by increasing the number of appropriate nests available, but by increasing the chances of discovering future laying opportunities. The parallels of farming

behaviour to infanticide observed in other species are notable, as sexually selected infanticide by males may also create additional breeding opportunities, advance the onset of those opportunities and improve the perpetrators ability to synchronize mating at the appropriate time.

In Chapter 4 I use radio-telemetry data on cowbirds to create multi-dimensional UDs that include a time as a dimension. I found that cowbirds were significantly more likely to be found around nesting sites when a nest was active suggesting that cowbirds prioritize their nest searching in areas where they are more likely to find a nest. I did not, however, find a difference in probability of occurrence depending on the developmental stage of a nest. Comparison of 2D spatial and 3D spatio-temporal breeding range estimates showed that strictly spatial analyses significantly overestimated overlap among individuals by an average of 20%, likely because individuals may occupy similar locations, but do so at different times. Analyses of behavioural observations indicated that female cowbirds tended to be solitary early in the day while nest searching, but associated with significantly larger groups of conspecifics as the day progressed. Also, aggressive interactions among females significantly decreased throughout the day suggesting that any territorial behaviour happens in the mornings when females are searching for host nests. Finally, using microsatellite DNA markers, I map individual female egg laying and show relatively little overlap among egg laying ranges. This is among the first practical applications of UDs that incorporate a time dimension and appears to provide a more realistic model of how cowbirds find host nests and interact with one another.

5.2 Cowbirds manipulate their hosts

Interactions between brood parasites and their hosts can serve as informative models for studying coevolutionary processes in the wild (Rothstein 1990). The complexity of the coevolutionary arms races between avian brood parasites and their hosts has become increasingly apparent in recent years (Feeney et al. 2014). Examples of parasitic adaptations and host counter adaptations have been found in every stage of the host nesting cycle including nest building, incubation, brooding and fledgling stages (Brooke and Davies 1988, Langmore et al. 2003. Welbergen and Davies 2009, De Mársico et al. 2012). Based on the results in this Thesis, farming behaviour by female cowbirds certainly appears to be another example of an adaptation to increase the transmission of the parasite. Many of the complexities of a farming strategy have yet to be elucidated. My simulation model (Chapter 2) predicts that farming should increase a cowbird's ability to find re-nesting attempts. Whether cowbirds actually alter their nest searching behaviour depending on past predation behaviour has yet to be determined. Future work may also identify counter adaptations in hosts to resist farming tactics (Chakra et al. 2017). For example, a 'farmed' host may lower the chances of their replacement nests being found by re-nesting further away or in more cryptic locations. It is clear that more research is needed into the subtleties and complexities of possible farming behaviour.

5.3 Specific challenges for studying cowbird predatory behaviour

In the field the most substantial challenge of studying cowbird predatory behaviour is not being able to directly observe females while they searched for host nests. Female cowbirds are

exceedingly cryptic in the mornings and even when equipped with a radio-telemetry transmitter it is often impossible to observe their behaviour directly (Swan personal observation). It is likely that this limitation has been the main reason why incidence of cowbirds causing total nest failure have been reported so rarely (Thompson et al. 1999; Pietz and Granfors 2000; Granfors et al. 2001). The use of cameras allowed me to document rarely observed behaviours of cowbirds while at a host nest. Specifically, cameras allowed me to provide valuable evidence that cowbirds act, not just as parasites, but as nest predators. In many cases, cowbirds would not have been suspected as the cause of nest failure if I was not monitoring with a camera. These video records were also invaluable because they allowed me to create the predator identification key discussed in Chapter 2. Even so, because so many failures resulted in empty nests that gave inconclusive evidence as to the identity of the nest predator, we are still unsure of the total impact of cowbird predatory behaviour in the wild. As cameras become increasingly miniaturized and memory rich I am confident that nest monitoring will be even more informative.

In Chapter 3 I carried out a series of experiments using captive cowbirds. This eliminated the need for monitoring cryptic females in the field, but presented other challenges. It took almost a full month before my birds in captivity would reliably approach the model nests used in the trials. Moreover, individuals needed a minimum of 24 hours to habituate to the flight chambers before each trial. Under these conditions the birds readily approached and attacked the nests with which they were presented and the experiments were a success. Even so I was unable to design experiments testing preferential laying patterns. In particular, I wished to test whether captive birds that attacked an artificial nest would return to parasitized ‘replacement’ nests as

would be expected under the farming hypothesis. Cowbirds typically require large aviaries that they need to be well habituated to if they are to lay in captivity (White et al. 2007, 2009).

5.4 Implications for future home range studies

The results in Chapter 4 clearly demonstrated the utility of including additional dimensions in resource selection and home range studies. In particular, my finding that the likelihood of finding a cowbird in an area varies with the temporal availability of a host nest suggests that time may be considered whenever resources are short-lived in the environment. Yet, similar to height and depth, time has hardly ever been considered when creating utilization distributions (Laver and Kelly 2008) and to my knowledge has never been practically incorporated simultaneously with spatial dimensions. That said, space use clearly varies with time and this variation may have important implications for spatial studies depending on the ecology and behaviour of the species being studied (Spencer et al. 1990; Keating and Cherry 2009; Cooper et al. 2014). Incorporating time into measures of spatial overlap may be especially important for measuring competition among species. For example, my results indicate a high degree of spatial overlap for cowbirds in two-dimensions, but significantly less in both space and time. Therefore accounting for time may help us to determine whether competition for resources is based on interference or exploitation.

The software packages used in Chapter 4 (ks: Duong 2007, adehabitat: Calenge 2006) allow for estimation of UDs in up to six dimensions. It should be possible, therefore, to include additional dimensions such as height/ depth or any other relevant variable, however, collection of the necessary data may present a challenge. A pivotal practical limitation in applying higher dimensional spatial models is the supposed “curse of dimensionality”, which states that the data requirements increase by at least one order of magnitude for each additional dimension of

interest (Silverman 1986). It is currently feasible to apply higher dimension UDs to larger species that can accommodate satellite tracking devices that automatically records many locations over time, however, this may not be possible with smaller species such as cowbirds. As technology gets smaller and more automated, this size limitation may no longer be relevant.

Bandwidth selection is often considered a problem when creating utilization distributions. In general, selecting bandwidths that are too small will create choppy home range estimates with false structure, and choosing bandwidths that are too large over-smooths estimates which can obscure important structure (White and Garrott 1992). Despite considerable research into optimal and objective bandwidth selection for two-dimensional UDs (Horne and Garton 2006; Zhang et al. 2006), almost no theory has been advanced for optimal bandwidth selection procedure for higher dimension UDs (Keating and Cherry 2009). In Chapter 4 I chose the smoothing factor fairly subjectively based largely on what seemed to fit with realistic cowbird movement. This procedure follows others that have suggested that the importance of choosing optimal bandwidth is often overstated (Scott 1992) and that it is often more appropriate to select bandwidths subjectively (Scott 1992; Silverman 1986). Even so, if we are to ensure objective results that are comparable among studies an objective bandwidth selection procedure for higher dimension utilization distributions may be needed in the future.

5.5 Implications for conservation

Several populations of passerines face many risks due to habitat loss and degradation (Samson et al. 2004; Askins et al. 2007; Benson et al. 2013). The expansion of energy infrastructure across North America, for example, may substantially contribute to population declines of grassland songbirds (Askins et al. 2007; Nasen et al. 2011). In order to develop suitable mitigation

strategies, it is first necessary to identify the specific threats associated with habitat fragmentation (Bernath-Plaisted et al. 2017). Increasing edge habitat has been associated with increases in brown-headed cowbird abundance (Howell et al. 2007) and overall parasitism rates (Johnson and Temple 1990; Patten et al. 2006). Such increases in cowbird abundance has been shown to lead to declining host species abundance (e.g. Smith et al. 2002) because cowbirds cause nest abandonment (18), reduce the number and quality of host young fledged (Burhans et al. 2000, Davies and Sealy 2000, Zanette et al. 2005) and may increase risk of predation (Mclaren and Sealy 2000). My findings indicate that cowbirds may also represent a substantial nest predator of passerines. Nest predation, in particular, is believed to be the most important cause of nest failure in birds. Thus, farming behaviour in cowbirds could amplify the potential threat of habitat loss on cowbird hosts and may have the potential to jeopardize populations already at risk.

5.6 Future directions

Further research is required to better understand the ecological, evolutionary and conservation implications of cowbird predatory behaviour. Clearly, we are still in the early stages of identifying the prevalence and reasons for these attacks. It is absolutely imperative that the identity of nest predators is reliably identified in other sites and that the frequency of cowbird attacks is established. Host species in other geographical areas certainly appear to endure cowbird predation (Arcese et al. 1996; Clotfelter and Yasukawa 1999; Hoover and Robinson 2007), but we are a far cry from confirming that such behaviour is ubiquitous.

If brown-headed cowbirds gain reproductive advantages by attacking late-stage nests one may expect to find predatory behaviour to be common among other brood parasites and

should be the subject of future study. Shiny cowbirds (*Molothrus bonariensis*) are also generalist brood parasites that regularly peck, puncture eggs from nest that they visit, but do not remove eggs as brown-headed cowbirds (Fraga 1998). It was recently demonstrated that, similar to my experiments in Chapter 3, shiny cowbirds puncture significantly more eggs in nests simulating complete clutches (containing 4 eggs) than those simulating incomplete clutches (containing one egg: Cossa et al. 2017). In either case, however, shiny cowbirds usually didn't puncture the complete clutch of eggs. These results were interpreted as cowbirds reducing nestling competition as opposed to enforcing host re-nesting (Cossa et al. 2017). However, hosts of shiny cowbirds will often abandon when not all eggs are destroyed (Massoni and Reboreda 1998; Tuero et al. 2007). Therefore, I strongly encourage future study of farming behaviour in other brood parasites such as shiny cowbirds.

Mafia behaviour in which brood parasites enforce future acceptance by destroying the nest contents of hosts that reject their eggs (Zahavi 1979) was first experimentally reported in great-spotted cuckoos (Soler et al. 1995) and later in brown-headed cowbirds (Hoover and Robinson 2007). The necessary mechanisms between possible farming and mafia behaviour are similar, however, according to each hypothesis, the reasons for causing nest failure are quite different. Whereas the farming hypothesis assumes that parasites cause failure to induce host re-nesting (Arcese et al. 1996), the mafia hypothesis assumes that loosing a nest after ejecting a parasitic egg will encourage future acceptance. This element of the mafia hypothesis where hosts are more likely to accept either within a season or across generations has not been demonstrated. In a series of elegant experiments Hoover and Robinson (2007) showed that nests where cowbird eggs were removed were preyed upon at high rates, non-parasitized nests were preyed upon at intermediate rates and parasitized nests in which the cowbird egg was not removed were hardly

preyed upon at all. While these results are consistent with the Mafia hypothesis, I suggest they could be explained by the farming hypothesis as well. Cowbirds destroy late-stage nests that they encounter (Chapter 3), avoid destroying nests they have parasitized (Chapter 2), and repeatedly visit nests that they have already laid in (Scott 1992). Therefore, the difference in predation rates in nests where a cowbird egg was removed and nests that were not parasitized reported by Hoover and Robinson (2007) may be explained by cowbirds simply encountering the previously parasitized nests more often. In either case, from the cowbird's perspective, they were attacking late-stage nests that did not contain one of their eggs. I believe that this is a more parsimonious explanation than the mafia hypothesis and should be the subject of future study.

5.7 Conclusions

This work is among the first to comprehensively evaluate the connection between nest predation and brood parasitism in brown-headed cowbirds. My research strongly supports the assertion of the farming hypothesis that cowbirds are common predators of their hosts, thereby creating future reproductive opportunities in a manner directly comparable to other infanticidal species. Moreover, my results suggest that cowbirds take advantage of these reproductive opportunities suggesting that their predatory behaviour may be an adaptation similar to other parasites that manipulate the behaviour of their hosts. Perhaps just as important, my work helps to elucidate how cowbirds use farming tactics to increase their reproductive output. In particular how they determine the developmental stage of a nest so that they can direct their attacks at late-stage nests no longer suitable for parasitism. Generally, I see the significance of my research as establishing whether farming is a potentially adaptive behaviour. If cowbirds derive a clear reproductive benefit from destroying host nests, as my research suggests, this behaviour is likely to be

commonplace, widespread and perhaps present in other species of brood parasites. Much of the controversy over whether cowbirds represent a significant conservation threat currently centers on the prevalence of their predatory behaviour (Rothstein and Peer 2005; Peer 2006; Peer et al. 2013; Zanette et al. 2007). Hence, my work will provide valuable insight into the mechanisms underlying the evolutionary refinement of brood parasitism and has immediate application to conservation strategy.

5.8 References

Arcese P, Smith JNM, Hatch MI. 1996. Nest predation by cowbirds and its consequences for passerine demography. Proc Natl Acad Sci. U.S.A. 93:4608-4611.

Askins RA, Chavez-Ramirez F, Dale BC, Haas CA, Herkert JR, Knopf F and Vickery PD. 2007. Conservation of grassland birds in North America: understanding ecological processes in different regions: 'Report of the AOU committee on conservation'. Ornithol. Monogr. 64:1-46.

Benson TJ, Chiavacci SJ and Ward MP. 2013 Patch size and edge proximity are useful predictors of brood parasitism but not nest survival of grassland birds. Ecol. App. 23:879-887.

Bernath-Plaisted J, Nenninger H and Koper N. 2017. Conventional oil and natural gas infrastructure increases brown-headed cowbird (*Molothrus ater*) relative abundance and parasitism in mixed-grass prairie. R. Soc. Open sci. 4:170036.

Brooke M and Davies NB. 1998. Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. Nature 335:630-632.

Burhans DE and Thompson FR III. 2000. Costs of parasitism incurred by two songbird species and their quality as cowbird hosts. Condor 102:364-373.

Calenge C. 2006. The package adehabitat for the R software: A tool for the analysis f space and habitat use by animals. Ecol. Modeling 197:516-519.

Chakra MA, Hilbe C and Traulsen A. 2017. Coevolutionary interactions between farmers and mafia induce host acceptance of avian brood parasites. R. Soc. Open Sci. 3:160036.

Clotfelter ED, Yasukawa K. 1999. Impact of brood parasitism by brown-headed cowbirds on red-winged blackbird reproductive success. Condor. 101:105-114.

Cooper NW, Sherry TW, and Marra PP. 2014. Modeling three-dimensional space use and overlap in birds. Auk. 131:681-693.

Cossa NA, Tuero DT, Reboreda JC and Fiorini VD. 2017. Egg pecking and puncturing behaviors in shiny and screaming cowbirds: effects of eggshell strength and degree of clutch completion. Behav. Ecol. Sociobiol. 71:60.

Davies SK and Sealy SG. 2000. Cowbird parasitism and nest predation in fragmented grasslands of southwestern Manitoba. In Ecology and management of cowbirds and their hosts: studies in the conservation of North American passerine birds (Smith JNM ed). Austin Texas: University of Texas Press.

De Mársico MC, Gantchoff MG and Reboreda JC. 2012. Host-parasite coevolution bryond the nestling stage? Mimicry of host fledglings by the specialist screaming cowbird. Proc. R. Soc. B. 279:3401-3408.

Duong T. 2007. ks: Kernel density estimation and kernel discriminant analysis for multivariate data in R. J. Stat. Software 21:1-16.

Feeney WE, Welbergen JA and Langmore NE. 2014. Advances in the study of coevolution between avian brood parasites and their hosts. Annu. Rev. Ecol. Evol. Syst. 45:227-246.

Fraga RM. 1998. Interactions of the parasitic screaming and shiny cowbirds (*Molothrus rufoaxillaris* and *M. bonariensis*) with a shared host, the bay-winged cowbird (*M. badius*). In: Rothstein SI, Robinson SK, editors, Parasitic birds and their hosts: studies in coevolution. Oxford University Press, New York, pp 173–193.

Granfors DA, Pietz PJ, Joyal LA. 2001. Frequency of egg and nestling destruction by female brown-headed cowbirds at grassland nests. Auk. 188:765-769.

Hoover JP and Robinson SK. 2007. Retaliatory mafia behavior by a parasitic cowbird favors host acceptance of parasitic eggs. Proc Nat Acad Sci U.S.A. 104:4479‑4483.

Horne JS and Garton EO. 2006. Likelihood cross-validation versus least squares cross-validation for choosing the smoothing parameter in kernel home-range analysis. J. Wildlife Manage. 70:641–648.

Howell CA, Dijak WD and Thompson FR III. 2007. Landscape context and selection for forest edge by breeding brown-headed cowbirds. Landsc. Ecol. 22:273-284.

Johnson RG and Temple SA. 1990. Nest predation and brood parasitism of tall grass prairie birds. J. Wildl. Manag. 54:106-111.

Keating KA and S Cherry S. 2009. Modeling utilization distributions in space and time. Ecology 90:1971-1980.

Langmore NE, Hunt S and Kilner RM. 2003. Escalation of a co-evolutionary arms race through host rejection of brood parasitic young. Nature 422:157-160.

Laver PN and Kelly MJ. 2008. A critical review of home range studies. J. Wildlife Manage. 72:290-298.

Massoni V and Reboreda JC. 1999. Egg puncture allows shiny cowbirds to assess host egg development and suitability for parasitism. Proc R Soc Lond B. 266:1871-1874.

Mclaren CM and Sealy SG. 2000. Are nest predation and brood parasitism correlated in yellow warblers? A test of the cowbird predation hypothesis. Auk 117:1056-1060.

Nasen LC, Noble BF and Johnstone JF. 2011. Environmental effect of oil and gas lease sites in a grassland ecosystem. J. Eviron. Manage. 92:195-204.

Patten MA, Shochat E, Reinking DL, Wolfe DH and Sherrod SK. 2006. Habitat edge, land management and rates of brood parasitism in tall grass prairie. Ecol Appl. 16:687-695.

Peer BD, Rivers JW and Rothstein SI. 2013. Cowbirds, conservation, and coevolution: potential misconceptions and directions for future research Chinese Birds 4:15-30.

Peer BD. 2006. Egg destruction and egg removal by avian brood parasites: adaptiveness and consequences. Auk 123:16-22.

Pietz PJ and Granfors DA. 2000. Identifying predators and fates of grassland passerine nests using miniature video cameras. J. Wildlife Manage. 64:71-87.

Rothstein SI. 1990. A model system for coevolution: avian brood parasitism. Ann. Rev. Ecol. Syst. 21:481-508.

Rothstein SI and Peer BD. 2005. Conservation solutions for threatened and endangered cowbird (*Molothrus spp*.) hosts: separating fact from fiction. Ornithol. Monogr. 57:98-114.

Samson FB, Knopf FL and Ostlie WR. 2004. Great plains ecosystems: past, present and future. Wildl. Soc. Bull. 32:6-15.

Scott, D. W. 1992. Multivariate density estimation: theory, practice, and visualization. John Wiley and Sons, New York, New York, USA.

Silverman BW. 1986. Density estimation for statistics and data analysis. Chapman and Hail, New York, New York, USA.

Smith JNM, Taitt MJ and Zanette L. 2002. Removing brown-headed cowbirds increases seasonal fecundity and population growth in song sparrows. Ecology. 83:3037-3047.

Soler M, Soler JJ, Martinez JG, Møller AP. 1995. Magpie host manipulation by great spotted cuckoos: evidence for an avian mafia? Evolution. 49:770-775.

Spencer SR, Cameron GN and Swihart RK. 1990. Operationally defining home range: Temporal dependence exhibited by hispid cotton rats. Ecology 71:1817–1822.

Thompson FR, Dijal W and Burhans DE 1999. Video identification of predators at songbird nests in old fields. Auk 116:259-264.

Tuero DT, Fiorini VD and Reboreda JC. 2007. Effects of shiny cowbird *Molothrus bonariensis* parasitism on different components of house wren *Troglodytes aedon* reproductive success. Ibis 149:521–529.

Welbergen JA and Davies NB. 2009. Strategic variation in mobbing as a front line of defence against brood parasitism. Curr. Biol. 19:235-240.

White GC and Garrott RA. 1992. Analysis of wildlife radio-tracking data. Academic Press, San Diego, California, USA.

White DJ, Ho L and Freed-Brown G. 2009. Counting chicks before they hatch: Female cowbirds can time readiness of a host nest for parasitism. Psycol Sci. 20:1140-1145.

White DJ, Ho L, de los Santos G and Godoy I. 2007. An experimental test of preferences for nest contents in an obligate brood parasite, *Molothrus ater*. Behav Ecol. 18:922-928.

Zahavi A. 1979. Parasitism and nest predation in parasitic cuckoos. Amer. Naturalist 113:157-159.

Zanette L, Haydon DT, Smith JNM, Taitt MJ and Clinchy M. 2007. Reassessing the cowbird threat. Auk. 124:210-223.

Zanette L, MacDougall-Shakleton E, Clinchy M and Smith JNM. 2005. Brown-headed cowbirds skew host offspring sex ratios. Ecology 86:815-820.

Appendix 1: Animal use protocol approval for Chapter 3

AUP Number: 2012-005
PI Name: Zanette, Liana
AUP Title: An Experimental Test Of Brown-headed Cowbird (*Molothrus ater*) Predatory Behaviour

Approval Date: 06/20/2012
Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "An Experimental Test Of Brown-headed Cowbird (*Molothrus ater*) Predatory Behaviour" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2012-005::1

. This AUP number must be indicated when ordering animals for this project.
. Animals for other projects may not be ordered under this AUP number.
. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee

Appendix 2: Python code for Monte Carlo simulation in Chapter 2

Appendix 2.1 Create the ‘cowbird’ object

```
class CowBird(object):
    'A class representing a cowbird'
    def __init__(self,yamlFile):
        self.interEggIntervalMean = yamlFile['interEggIntervalMean']
        self.interEggIntervalStdDev = yamlFile['interEggIntervalStdDev']
        self.initEggTimer()
        self.totalEggs = 0
        self.wastedEggs = 0
        self.cowbirdType = yamlFile['cowbirdType']
        self.cowbirdUpdatedProbabilityOfBeingFoundForParasitism =
            yamlFile['cowbirdUpdatedProbabilityOfBeingFoundForParasitism']

    def initEggTimer(self):
        self.eggTimer =
            max(1,round(random.normalvariate(self.interEggIntervalMean,self.interEggInter
            valStdDev)))

    def step(self):
        self.eggTimer-=1
        layEgg = False
        if self.eggTimer == 0:
            self.initEggTimer()
            layEgg = True
            self.totalEggs = self.totalEggs + 1
        return layEgg

    def nextEggTime(self,currentTime):
        return currentTime + self.eggTimer

# conditions for the non-farmer cowbird
simulation:
  seasonLength: 120
  numberOfNests: 5
  interEggIntervalMean: 2.1
  interEggIntervalStdDev: 0.3
  # Type of cowbird: Standard, SimpleFarmer, or AdvancedFarmer
```

```
  cowbirdType: Standard
  cowbirdUpdatedProbabilityOfBeingFoundForParasitism: 0.05

# conditions for the simple farming cowbird
simulation:
  seasonLength: 120
  numberOfNests: 5
  interEggIntervalMean: 2.1
  interEggIntervalStdDev: 0.3
  # Type of cowbird: Standard, SimpleFarmer, or AdvancedFarmer
  cowbirdType: SimpleFarmer
  cowbirdUpdatedProbabilityOfBeingFoundForParasitism: 0.05

# conditions for the advanced farming cowbird
simulation:
  seasonLength: 120
  numberOfNests: 5
  interEggIntervalMean: 2.1
  interEggIntervalStdDev: 0.3
  # Type of cowbird: Standard, SimpleFarmer, or AdvancedFarmer
  cowbirdType: AdvancedFarmer
  cowbirdUpdatedProbabilityOfBeingFoundForParasitism: 0.9
```

Appendix 2.2 Create the ‘nest’ object

```
class Nest(object):
    'A song sparrow nest and associated state'
    def __init__(self, nestId, nestyaml):
        if not type(nestyaml) is dict:
            # Load the yaml file
            with open(nestyaml) as nesty:
                nestyaml = yaml.load(nesty.read())
        # Now nestyaml is a yaml object
        theNest = nestyaml['nest']

        statesYaml = theNest['states']
        states={}
        # This is a trick for building structures from the
        # YAML file.
```

```
    for key, value in statesYaml.items():
        states[key] = util.Struct(**value)
    # Now "states" is a dictionary. The key
    # is the state name (as a string), the
    # value is a struct with the fields necessary
    # for the simulation:
    #   durationMean:                       4
    #   durationStdDev:                     0
    #   nextStateName:                      Building
    #   probabilityOfBeingPredated:             0.0
    #   probabilityOfBeingFoundForParasitism:     0.0
    #   probabilityOfBeingFoundForCowbirdAttack:  0.0
    #   resetProbabilitiesOnTransition          True

    # Set up the internal state of the object
    self.initialState = theNest['initialState']
    self.initialStates = states
    self.parasitized=False
    self.isParasitized=False
    self.nestId = nestId
    self.stateName = self.initialState

def transitionIntoInitialState(self):
    return self.doStateTransition(self.initialState, True, "InitialState")

def copyStates(self):
    #return copy.deepcopy(self.initialStates)
    states = {}
    for key, value in self.initialStates.iteritems():
        states[key] = util.Struct( **(value.__dict__) )
    return states
def doStateTransition(self, stateName, resetProbabilities, reason):
    'Sets the state and does bookkeeping'
    if resetProbabilities:
        self.states = self.copyStates()

    transition = util.StateTransition(self.nestId, self.stateName, stateName, reason,
         self.isParasitized)

    self.stateName = stateName
```

```
    self.state = self.states[stateName]
    self.stateTime =
        max(1,round(random.normalvariate(self.state.durationMean,self.state.durationSt
        dDev)))

    # Hardcode the reset of parasitization to Idle transtions.
    if self.stateName == "Idle":
        self.isParasitized=False

    return transition

def checkForPredation(self):
    transition = None

    # Check for predation
    if self.state.probabilityOfBeingPredated > np.random.rand():
        transition = self.doStateTransition( 'Idle', True, "PredatorAttack" )
    return transition

def step(self, seasonOver):
    'A function to process one normal timestep'

    # If the season is over and the state is in idle,
    # do nothing.
    if seasonOver and self.stateName == 'Idle':
        return

    transition = None

    # Decrement the time and do a state transition if necessary
    self.stateTime -= 1
    if self.stateTime == 0:
        # This is a timeout. Process the state transition
        transition = self.doStateTransition( self.state.nextStateName,
         self.state.resetProbabilitiesOnTransition, "Timeout" )

    return transition
```

Appendix 2.3 Running a single simulation

```
class Simulation(object):
    'An object representing a single simulation'
    def __init__(self, simYaml, nestYaml):
        # Now yamlFile is a dictionary
        self.yaml = simYaml['simulation']

        nests = []
        for i in range(0,self.yaml['numberOfNests']):
            nests.append( nest.Nest(i, nestYaml) )

        self.cowbird = cowbird.CowBird(self.yaml)

        self.nests = nests
        self.seasonLength = self.yaml['seasonLength']
        self.day=0
        self.transitions = []
        self.cowbirdEggs = []
        for n in self.nests:
            transition = n.transitionIntoInitialState()
            self.transitions.append(transition)


    def step(self):
        'Do one step of the simulation'

        # A new day dawns!
        self.day += 1

        # The cowbird's internal clock advances.
        isLaying = self.cowbird.step()

        egg = None
        if isLaying:
            # Create an egg
            egg = util.CowBirdEgg(self.day,None)

        # The cowbird visits the nests in random order
        nestIndices = range(len(self.nests))
        random.shuffle(nestIndices)
```

```
    for idx in nestIndices:
        nest_i = self.nests[idx]

        # The cowbird should move first.
        # If the cowbird lays its egg,
        # the nestid will be set
        transition, egg = self.cowbirdExaminesNest(nest_i, egg)

        # Now we check for predation
        if transition is None:
            transition = nest_i.checkForPredation()

        # Then we step the nest...this checks for predation,
        # then it checks timeout values.
        if transition is None:
            transition = nest_i.step(self.isSeasonOver())

        # Here we can do statistics on transitions.
        # Each day can create exactly one transition
        # Either the result of a timeout, or a predation
        # event
        if not transition is None:
            # Store the current day
            transition.day = self.day
            self.transitions.append(transition)

    if not egg is None:
        self.cowbirdEggs.append(egg)
        if not egg.isLaid():
            # The cowbird was not able to find a nest for this egg...the egg is wasted
            self.cowbird.wastedEggs = self.cowbird.wastedEggs + 1

def cowbirdExaminesNest(self, nest_i, egg):
    transition = None
    if (not egg is None and not egg.isLaid()) and \
       not nest_i.isParasitized:
        # This nest may be parasitized. Check if the cowbird can find the nest
        draw = random.random()
        if draw < nest_i.state.probabilityOfBeingFoundForParasitism:
```

```
            # print "Probability: {0}, random draw:
        {1}".format(nest_i.state.probabilityOfBeingFoundForParasitism,draw)
            # The nest is parasitized.
            nest_i.isParasitized = True
            # The egg is used up
            egg.nestId = nest_i.nestId

    # Check if the cowbird attacks the nest.
    if not self.cowbird.cowbirdType == "Standard":
        if not nest_i.isParasitized:
            draw = random.random()
            if draw < nest_i.state.probabilityOfBeingFoundForCowbirdAttack:
                # This nest is attacked!
                if self.cowbird.cowbirdType == "SimpleFarmer":
                    transition = nest_i.doStateTransition( "Idle", True, "CowbirdAttack" )
                elif self.cowbird.cowbirdType == "AdvancedFarmer":
                    # This is the advanced farmer...
                    transition = nest_i.doStateTransition( "Idle", True, "CowbirdAttack" )
                    # increase the probability of finding the nest during parasitism
                    nest_i.states['Laying'].probabilityOfBeingFoundForParasitism =
        self.cowbird.cowbirdUpdatedProbabilityOfBeingFoundForParasitism

        nest_i.states['IncubatingAppropriate'].probabilityOfBeingFoundForParasitism =
        self.cowbird.cowbirdUpdatedProbabilityOfBeingFoundForParasitism
                else:
                    raise RuntimeError("Unknown cowbird type {0}. Try 'Standard',
        'SimpleFarmer', or 'AdvancedFarmer'")

    return (transition, egg)

def isSeasonOver(self):
    return self.day >= self.seasonLength

def isSimOver(self):
    simOver = False
    if self.isSeasonOver():
        simOver = True
        # Check if the simulation is over
        for n in self.nests:
            if not n.stateName == 'Idle':
```

```
            simOver = False

        return simOver

    def runFullSim(self):
        while not self.isSimOver():
            self.step()

    def countNestsThatStartBuilding(self):
        return len([ t for t in self.transitions if t.stateTo == 'Building' ])

    def countNestsThatArePredatorized(self, isParasitized=None):
        return len([ t for t in self.transitions if t.reason == 'PredatorAttack' and (isParasitized
            is None or t.isParasitized == isParasitized) ])

    def countCowbirdAttacks(self):
        return len([ t for t in self.transitions if t.reason == 'CowbirdAttack' ])


    def countNestsThatSucceed(self, isParasitized=None):
        return len([ t for t in self.transitions if  t.reason == 'Timeout' and t.stateTo == 'Idle'
            and (isParasitized is None or t.isParasitized == isParasitized) ])

    def countParasitizedNests(self):
        return len([ e for e in self.cowbirdEggs if not e.nestId is None ])

    def countEggs(self):
        return len( self.cowbirdEggs )
```

Appendix 2.4 Run experiment using multiple simulations for multiple cowbird types

```
# All simulations use the same nest definition file.
with open('nest.yaml') as yamldoc:
    nestYamlOriginal = yaml.load(yamldoc.read())

# Loop through the farmers
yamls = ['simulationStandard.yaml', 'simulationSimpleFarmer.yaml',
            'simulationAdvancedFarmer.yaml']
# Loop through different predation levels
```

```
# np.linspace( startingValue, endingValue, numberOfSteps)
predationLevels = np.linspace(0.0,0.1,11)
# Number of trials to run for each combination (yaml, predationLevel)
NUM_TRIALS=10

idxFarmerType = 0
idxPredationLevel = 1
idxTotalNests = 2
idxTotalNestsStd = 3
idxTotalParasitizedNests = 4
idxTotalParasitizedNestsStd = 5
idxTotalNestsFledged = 6
idxTotalNestsFledgedStd= 7
idxTotalParasitizedNestsFledged = 8
idxTotalParasitizedNestsFledgedStd = 9
idxTotalNonparasitizedNestsFledged = 10
idxTotalNonparasitizedNestsFledgedStd = 11
idxTotalNestsPredated = 12
idxTotalNestsPredatedStd = 13
idxTotalParasitizedNestsPredated = 14
idxTotalParasitizedNestsPredatedStd = 15
idxTotalNonparasitizedNestsPredated = 16
idxTotalNonparasitizedNestsPredatedStd = 17
idxTotalCowbirdAttacks = 18
idxTotalCowbirdAttacksStd = 19
idxTotalEggs=20
idxTotalEggsStd=21
idxTotalWastedEggs=22
idxTotalWastedEggsStd=23
idxTotalLaidEggs=24
idxTotalLaidEggsStd=25
tableSize = 26

output = np.zeros([len(yamls)*len(predationLevels), tableSize])

for yamlIdx in range(0,len(yamls)):
    yam = yamls[yamlIdx]
    print "Processing {0}".format(yam)
    with open(yam) as yamldoc:
        simYaml = yaml.load(yamldoc.read())
```

```
for predationLevelIdx in range(0,len(predationLevels)):
    nestYaml = copy.deepcopy(nestYamlOriginal)
    outputRow = yamlIdx * len(predationLevels) + predationLevelIdx

    probabilityOfBeingPredated = predationLevels[predationLevelIdx]
    print "\tProcessing predation level of {0}".format(probabilityOfBeingPredated)
    theNest = nestYaml['nest']
    statesYaml = theNest['states']
    # Update the background predation levels
    for key, value in statesYaml.items():
        if value['probabilityOfBeingPredated'] > 0.0:
            value['probabilityOfBeingPredated'] = probabilityOfBeingPredated
    # Test...
    for key, value in statesYaml.items():
        if value['probabilityOfBeingPredated'] > 0.0:
            assert(value['probabilityOfBeingPredated'] == probabilityOfBeingPredated)

    totalNests = []
    totalParasitizedNests=[]
    totalNestsFledged=[]
    totalParasitizedNestsFledged = []
    totalNonparasitizedNestsFledged = []
    totalNestsPredated = []
    totalParasitizedNestsPredated = []
    totalNonparasitizedNestsPredated = []
    totalCowbirdAttacks = []
    totalEggs = []
    totalEggsWasted = []
    totalEggsLaid = []

    for i in range(0,NUM_TRIALS):
        sim = simulation.Simulation(simYaml, nestYaml)
        sim.runFullSim()
        # Accumulate statistics
        totalNests.append(sim.countNestsThatStartBuilding())
        totalParasitizedNests.append(sim.countParasitizedNests())
        totalNestsFledged.append(sim.countNestsThatSucceed())
```

```
            totalParasitizedNestsFledged.append(sim.countNestsThatSucceed(isParasitized=
            True))

            totalNonparasitizedNestsFledged.append(sim.countNestsThatSucceed(isParasitiz
            ed=False))
        totalNestsPredated.append(sim.countNestsThatArePredatorized())

            totalParasitizedNestsPredated.append(sim.countNestsThatArePredatorized(isPar
            asitized=True))

            totalNonparasitizedNestsPredated.append(sim.countNestsThatArePredatorized(is
            Parasitized=False))
        totalCowbirdAttacks.append(sim.countCowbirdAttacks())
        totalEggs.append(sim.countEggs())
        totalEggsWasted.append(sim.countWastedEggs())
        totalEggsLaid.append(sim.countLaidEggs())

    lists=[ totalNests, totalParasitizedNests, totalNestsFledged,
            totalParasitizedNestsFledged, totalNonparasitizedNestsFledged,
            totalNestsPredated, totalParasitizedNestsPredated,
            totalNonparasitizedNestsPredated, totalCowbirdAttacks, totalEggs,
            totalEggsWasted, totalEggsLaid ]

    # Write the output for this yaml file
    output[outputRow,idxFarmerType] = yamlIdx
    output[outputRow,idxPredationLevel] = probabilityOfBeingPredated
    offset = 2
    for idx in range(0,len(lists)):
        vals = np.array(lists[idx])
        mean = np.mean(vals)
        std  = np.std(vals)
        output[outputRow, offset + (2*idx)] = mean
        output[outputRow, offset + (2*idx) + 1] = std


# Save the output
np.savetxt('output.csv', output, delimiter=', ')
```

Appendix 3: Actual values from Monte Carlo simulation in Chapter 2

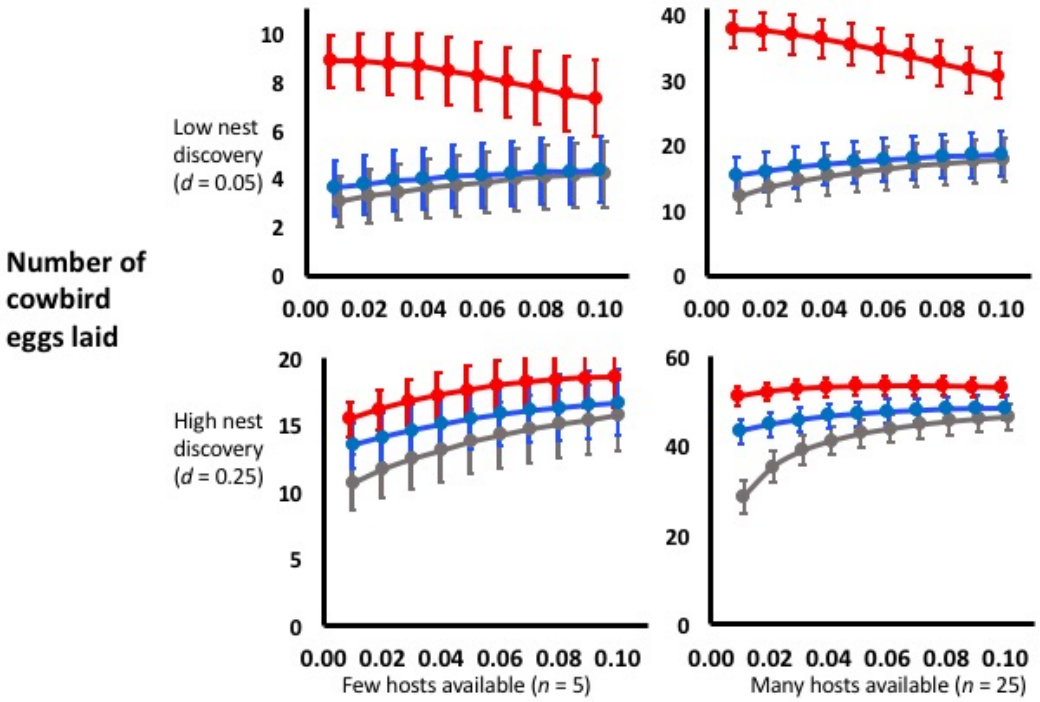

Figure App3.1: The number of cowbird eggs laid by non-farmers (grey), simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*).

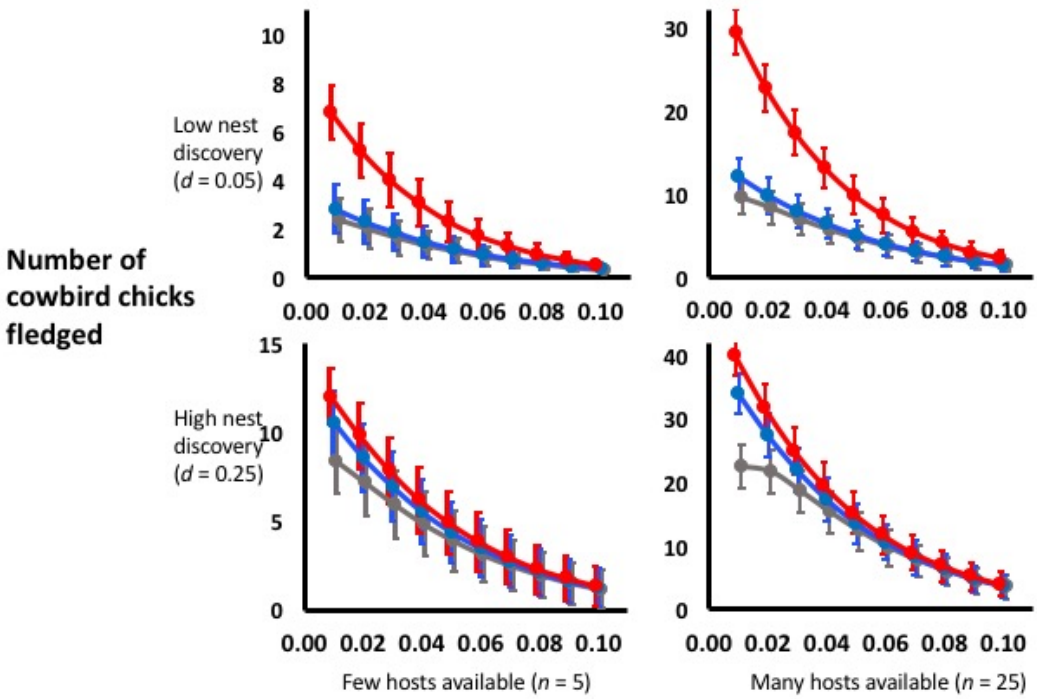

Figure App3.2: The number of cowbird chicks fledged by non-farmers (grey), simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*).

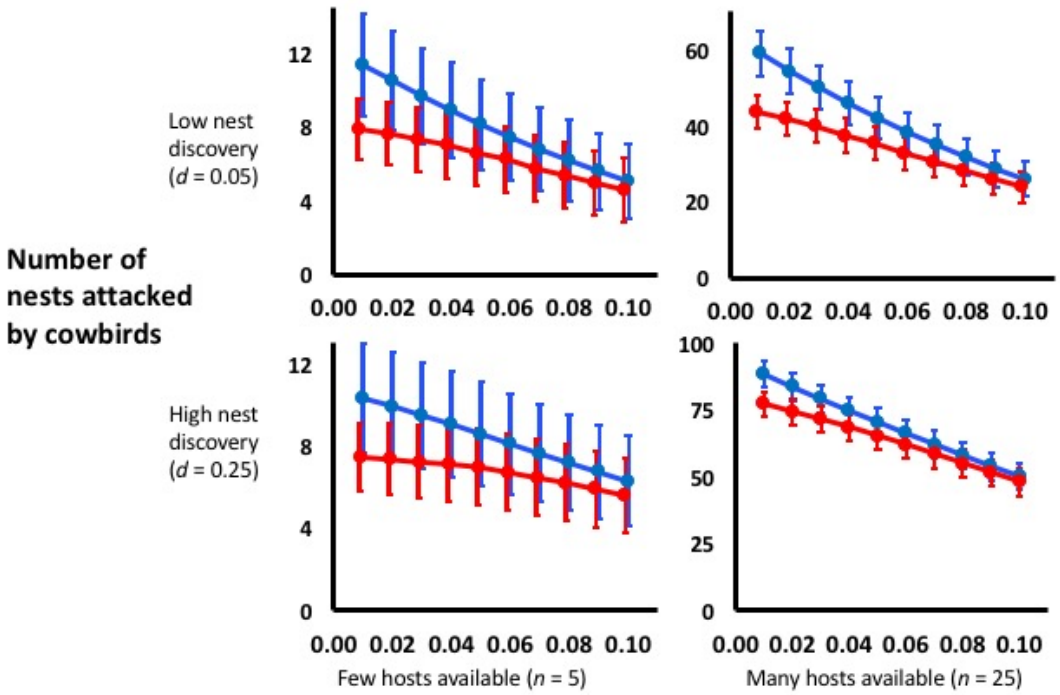

Figure App3.3: The number of attacks on host nests by simple (blue) and advanced farmers (red). Values are plotted as a function of the daily probability of failure due to non-cowbird predators (*a*) using different illustrative high and low values of the number of hosts available (*n*) and the daily probability that a nest will be discovered by a cowbird (*d*).

Curriculum Vitae

David C. Swan

EDUCATION

2009-present (expected completion March 2018)

Ph.D. Department of Biology, University of Western Ontario (UWO), London

Thesis: Nest predation by Brown-headed Cowbirds (*Molothrus ater*).

Supervisor: Professor Liana Zanette

2006-2008

M.Sc. Department of Biological Sciences, University of Manitoba, Winnipeg

Thesis: Larval recognition in two host species (*Temnothorax longispinosus* and *T. ambiguus*) of the slave-making ant *Protomognathus americanus*.

Supervisor: Professor James Hare

2002-2006

B.Sc. (Honours) Department of Ecology, University of Manitoba, Winnipeg

Thesis: Signaler age/syntax effects on the perceived urgency of Richardson's ground squirrel (*Spermophilus richardsonii*) alarm calls.

Supervisor: Professor James Hare

Awards, Distinctions and Fellowships

2012: Queen Elizabeth II Graduate Scholarship in Science and Technology
2012: Biology Graduate Research Forum 1st place award short talk, UWO
2011: Biology Graduate Research Forum 3rd place award long talk, UWO
2011: Graduate Student Travel Award, Department of Biology, UWO
2010: Frank M. Chapman Research Grant
2010: Graduate Thesis Research Award, UWO
2009: American Ornithologists' Union Research Award
2009: Society of Canadian Ornithologists James L. Baillie Student Research Award
2008-2011: NSERC Alexander Graham Bell Canada Graduate Scholarship (CGS)
2008: Lubinsky Memorial Scholarship
2007-2008: NSERC Postgraduate Scholarship
2007: Roger Evans Memorial Scholarship
2006-2007: Manitoba Graduate Scholarship
2006: NSERC Undergraduate Student Research Award
2006: H. E. Welch Award of Excellence in Zoology, University of Manitoba

2005-2006: Dean's Honour List, University of Manitoba
2003-2004: Deans Honour List, University of Manitoba
2002-2003: UMSU Scholarship; University 1 Honour List, University of Manitoba
2002: University of Manitoba Students' Union Scholarship

Relevant Work, Volunteer, and Teaching Experience

2012: Teaching Assistant for Ecology of Populations (BIOL 3440), Department of Biology, University of Western Ontario

- Taught hands-on techniques of population biology for its practical application

2009-2012: Teaching Assistant for Conservation Biology (BIOL 3442), Department of Biology, University of Western Ontario

- Facilitated group discussions, assisted with experimental design of environmental assessment projects

2009-2011: Teaching Assistant for Scientific Method in Biology (BIOL 2290), Department of Biology, University of Western Ontario

- Provided academic help to students, marked assignments

2008: Prairie Universities Biological Symposium (PUBS) organizing committee

- Participated in fundraising, organization and logistics of conference

2006-2008: Teaching Assistant II for Animal Behaviour (ZOOL 3100), Department of Biological Science, University of Manitoba

- Assisted students with experimental design and marked term projects

2005-2008: Teaching Assistant I for Introductory Invertebrate Zoology (ZOOL 2600), Department of Biological Science, University of Manitoba

- Provided academic help to students, presented pre- and post-lab review, created and marked lab exams

2005: Field Biological Researcher, Department of Biological Science, University of Manitoba

- Designed and executed a playback experiment and interpreted and presented results

PUBLICATIONS

PUBLICATIONS IN REFEREED JOURNALS

Swan, D.C., Zanette, L., and Clinchy, M. 2015. Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis. *Animal Behaviour* 105:29-35.

Swan, D.C. and Hare, J.F. 2012. Larval recognition by *Temnothorax longispinosus* and *T. ambiguous* hosts of the slave making ant *Protomognathus americanus* revisited: colony-level referent ensures conspecific preference. *Insectes Sociaux* 59:511-517.

Swan, D.C. and Hare, J.F. 2008. Signaler and receiver ages do not affect responses to Richardson's ground squirrel alarm calls. *Journal of Mammalogy* 89:889-894.

Swan, D.C. and Hare, J.F. 2008. The first cut is the deepest: primary syllables of Richardson's ground squirrel, *Spermophilus richardsonii*, repeated calls alert receivers. *Animal Behaviour* 76:47-54.

PUBLICATIONS IN NON-REFEREED JOURNALS

Swan, D.C. 2012. Cowbirds 'farm' other birds. *Riversong: newsletter of the Lower Grand River land trust*. 18(2).

Presentations

Swan, D.C., Zanette, L., and Clinchy, M. 2013. Brown-headed Cowbirds 'farm' their hosts. Talk presented at the American Ornithologists' Union and Cooper Ornithological Society meeting, 2013.

Swan, D.C., Zanette, L., and Clinchy, M. 2012. Brood parasitic cowbirds preferentially attack late-stage nests. Talk presented at the Biology Graduate Research Forum, University of Western Ontario, 2012.

Swan, D.C. 2012. The 'farming' behaviour of brown-headed cowbirds. Talk presented at the 3rd Annual Ruthven's for the Birds Festival, Cayuga, Ontario.

Swan, D.C., Zanette, L., and Clinchy, M. 2011. Spatial patterns of egg laying and nest predation by brown-headed cowbirds (*Molothrus ater*). Talk presented at the Animal Behaviour annual meeting.

Swan, D.C., Zanette, L., and Clinchy, M. 2011. Cowbirds hold stable egg laying ranges. Talk presented at the Biology Graduate Research Forum, University of Western Ontario, 2011.

Swan, D.C. 2010. Brown-headed cowbird parasitism and songbird conservation. Talk presented to the Otter Valley Naturalists Club in Port Burwell, Ontario

Swan, D.C. 2008. Larval recognition in two host species (*Temnothorax longispinosus* and *T. ambiguus*) of the slave-making ant *Protomognathus americanus.* Talk presented at the Prairie Universities Biological Symposium (PUBS), 2008.

Hare, J.F., Swan, D.C., Attygalle, A.B., and Eisner, T. 2007. Pump up the jam: chemical manipulation of slave brood preferences by slave-making ants. Talk presented at the Canadian Society of Zoologists Annual Meeting, 2007.