

BEHAVIORAL ECOLOGY OF JUVENILE BROWN-HEADED COWBIRDS: IMPLICATIONS FOR
THE EVOLUTION AND MAINTENANCE OF AVIAN BROOD PARASITISM

BY

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DISSERTATION

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ABSTRACT

Avian interspecific brood parasites are species that forego parental care and rely on heterospecifics to raise parasitic offspring. Host-parasite coevolutionary arms races and the constraints associated with a non-parasitic bird transitioning to a parasite likely contribute to the rarity of this reproductive strategy (ca. 1% of species). For my dissertation, I conducted research on juvenile brown-headed cowbirds (*Molothrus ater*) to elucidate the factors that contribute to the evolution and persistence of avian brood parasitism. To avoid adopting the behaviors and mate choice preferences (sexual imprinting) of the foster species, juvenile cowbirds could theoretically follow adult female cowbirds away from the host's care to avoid mis-imprinting on their host's phenotype. Using an automated radio telemetry system to identify the presence-absence (every 1-2 minutes) within a forest for juvenile and adult female cowbirds, I concluded that juveniles typically depart the host's care solitarily. Therefore, innate segregation behaviors likely facilitate the avoidance of mis-imprinting on the host's phenotype. Next, I used a prothonotary warbler (*Protonotaria citrea*) nest box dataset, comprising 21 years (1994-2014), to demonstrate that fledgling cowbirds provide reliable information indicating host quality that is used in future egg-laying decisions of adult female cowbirds. Both site-wide cowbird productivity and experimental egg removal indicated that reproductive performance information of cowbirds affects the probability of parasitism during the following nesting attempts within and between years. This result suggests that by avoiding poor hosts (e.g. egg rejecters), cowbirds could increase their reproductive success and delay the development of anti-parasite strategies in hosts. Finally, I demonstrated that fledging a cowbird in the first brood increases the likelihood for female warblers to initiate a second brood within the same season (i.e. double-brooding). I established that non-parasitized warblers respond to natural fecundity reduction (i.e. hatching failure and nestling death) with increased frequency of double-brooding. Then, I showed that raising a brood parasite induces compensatory double-brooding for female warblers by parasite-induced fecundity reduction. Consequently, hosts can recoup some fitness lost to infection (i.e. compensatory double-brooding) and parasites can enhance transmission by gaining additional parasitism opportunities, further delaying the development of anti-parasite strategies and avoiding the initiation of host-parasite arms races.

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CHAPTER 1

GENERAL INTRODUCTION

Interactions between parasites and their hosts play important roles in natural ecosystems, from influencing population dynamics to increasing genetic diversity through coevolutionary processes (e.g. Yoder and Nuismer 2010; Thompson 2014). Reciprocal host adaptations that counteract the fitness costs induced by parasites and the ensuing counter-adaptations developed by parasites (i.e. arms race) are predicted to increase variability among populations while reducing the extinction risk for host and parasites—the ‘Red Queen hypothesis’ (Van Valen 1973; Lively and Dybdahl 2000; but see Vermeij and Roopnarine 2013; Quental and Marshall 2013). However, transitioning from a non-parasitic to a parasitic life-style requires overcoming significant constraints (Poulin 2011) and the defense strategies of hosts can lead to the extinction of parasites (May and Anderson 1990). How animals switch to a parasitic lifestyle and coexist with hosts in the face of resistant strategies within natural populations remains poorly understood (Anderson and May 1978; Sheldon and Verhulst 1996; Späth et al. 2003; Dunn et al. 2009).

Obligate brood parasitism in birds, (species that manipulate heterospecific parent(s) into raising parasitic offspring) is a ‘model system’ for the study of coevolution in nature (reviewed in Rothstein 1990; Feeney et al. 2014; Soler 2014). Naturalists since Aristotle (4th century) have marveled at how parasites, such as the cuckoo, rely on surrogate species nearly half their size for nest building, incubation and feeding of parasitic offspring to complete reproduction (Davies and Brooke 1988). The fitness costs incurred by hosts and the development of anti-parasite strategies are the most studied consequences of brood parasitism, such as the host’s vigilance at the nest (e.g. Welbergen and Davies 2009; Feeney et al. 2012), recognition and rejection or abandonment of parasitic eggs (e.g. Rothstein 1990; Brooke and Davies 1988; Spottiswoode and Stevens 2010) and recognition of parasitic chicks (e.g. Langmore et al. 2003; Grim 2007). Consequently, to counteract the host’s defenses, parasites develop adaptations to counteract the hosts defenses such as hawk mimicry (e.g. Davies and Welbergen 2008), mimetic eggs (e.g. Lahti and Lahti 2002; Stevens et al. 2013), ejection of host eggs (Kilner and Langmore 2011) or by

killing host nestmates (e.g. Spottiswoode and Koorevaar 2011). Host's adaptations (Marchetti 1992) and reciprocal counter-adaptations by parasites are costly to produce (Anderson et al. 2009) and an escalating arms race could result in extinction for either the parasite or host (Dawkins and Krebs 1979). Therefore, specific strategies that reduce the costs of parasitism for the parasite or host may be beneficial for both parties in some situations (reviewed in Svensson and Råberg 2010; Medina and Langmore 2015).

More than 90% of birds provide parental care to their offspring (Cockburn 2006), yielding abundant opportunities for reproductive parasitism to evolve. Within birds, brood parasitism has evolved independently at least 7 times, each from a non-parasitic ancestor (Yom-Tov and Geffen 2006). Brood parasites, are phylogenetically widespread, having been identified in insects, fish and birds (Davies 2000). Yet, given the obvious reproductive advantages to brood parasitism, this breeding strategy is relatively rare in all taxa; limited to only a few genera of ants, fish and < 1 % of all bird species (Payne 1977; Davies et al. 1989). Why is brood parasitism so rare? Two main constraints likely contribute to the rarity of interspecific avian brood parasitism: mis-imprinting on the host (Slagsvold 1998) and anti-parasite defenses developed by hosts from coevolutionary arms races (Rothstein 1975).

Nearly every avian species and many mammals rely on sexual imprinting, using parents and siblings to form recognition templates for species recognition and mate selection (Irwin and Price 1999, ten Cate and Vos 1999). Although some form of imprinting must be important for brood parasites to remain in the care of their host parents and then subsequently parasitize the same host species (Payne et al. 2000), imprinting also acts as an important constraint in the evolution and maintenance of brood parasitism (Slagsvold and Hansen 2001). For example, in studies that switch the offspring of two bird species (cross-foster), the juveniles fail to recognize conspecifics and, subsequently, adopt behaviors of and attempt to mate with the foster species (ten Cate and Vos 1999; Slagsvold and Hansen 2001). Investigations of the behavioral ecology of juvenile brood parasites would help reveal how they avoid mis-imprinting on their foster species and recognize conspecifics (ten Cate and Vos 1999; Davies 2000; Hauber et al. 2001).

Juvenile brood parasites may also enhance our understanding of the persistence of host-parasite associations. Host quality varies widely for brood parasites, where the ability to raise parasite offspring is dependent upon the host's rejection strategies (Rothstein 1990; Soler 2014) or the ability to incubate parasitic eggs and feed the young (Middelton 1977; Grim et al. 2011). While the costs of reproduction are minimized by forgoing parental care, brood parasites are limited by seasonal decline in host availability (Curson and Mathews 2003) and the number of eggs they can produce (Holford and Roby 1993). Therefore, selection should favor cognitive abilities of parasites to choose the highest quality host(s) available. When reproductive success is predictable, reproductive performance is among the most reliable sources of biological information, enabling individuals to enhance fitness by minimizing uncertainty associated with breeding in heterogeneous environments (Danchin et al. 2004; Schmidt et al. 2010). The outcomes of past parasitic attempts may provide reliable information indicating the host's future reproductive potential. Therefore, female parasites could enhance their reproductive success by adjusting host preferences in response to their own (personal information) and other cowbirds (social information) experiences. Theoretically, host selectivity would impact population and evolutionary dynamics (e.g. Kawecki 1998), where increasing parasitism pressure for good hosts would select for anti-parasite strategies, while conversely, avoiding poor hosts with parasite-resistant strategies would delay or decrease the effectiveness of resistance (Kelly 1987; Marchetti 1992; Takasu 1998).

Parasitic strategies that reduce the fitness cost to hosts (i.e. virulence), yet manipulate the host's phenotype to enhance the parasite's fitness and transmission, may further reduce or delay the development of host resistance. Most organisms compensate for fitness losses by altering life-history traits (Metcalf and Monaghan 2001). In turn, parasites could mimic what induces a compensatory response (e.g. reducing fecundity) in hosts and achieve fitness benefits if the response to infection increases the likelihood of transmission for the parasite (Lefèvre et al. 2009; Lefèvre et al. 2008; Thomas et al. 2012). Because compensation would also enable hosts to recoup some parasite-induced fitness loss, the development of host resistance to parasitism would be diminished (Lefèvre et al. 2008; Svensson and Råberg 2010).

In my thesis, I focused on the behaviors of juvenile brown-headed cowbirds (*Molothrus ater*) and the effects of cowbird fledglings on the reproductive decisions of adult female cowbirds and those of a high quality host, the prothonotary warbler (*Protonotaria citrea*). Widespread throughout most of North America, the brown-headed cowbird has been documented laying eggs in the nests of over 200 species, while successfully fledging offspring from ~ 140 host species (Lowther 1993). Adult cowbirds forage socially in pastures and agriculture, and subsequently parasitize the songbirds inhabiting the surrounding landscape. Juvenile cowbirds are cared for by foster parents for 20-30 days after fledging and typically raised in the absence of conspecific nestmates (Woodward 1983). Common in the swamps and flooded bottomland forests throughout the southeastern US, the prothonotary warbler is the only species to nest in artificial nest boxes that is regularly parasitized by cowbirds (Petit 1999). By efficiently gaining access to parasitized nests, and the apparent lack of anti-parasite defenses of prothonotary warblers (Hoover 2003), the Cache River nest box study system provides a unique opportunity to explore the behaviors of juvenile brood parasites and their effects on the future reproductive decisions of parasites and hosts.

In Chapter 2, I investigated the movements of adult female and post-fledging cowbirds to identify potential behaviors that enable juveniles to avoid imprinting on their hosts and locate conspecifics. While innate predispositions for some conspecific traits likely guide conspecific recognition (King and West 1977), much of the species-specific songs and behaviors for cowbirds are acquired through experience with conspecifics (Göth and Hauber 2003). For example, captive cowbirds adopted the behaviors and attempted to mate with canaries after associating for 1 year, indicating that brood parasites can sexually imprint on hosts if they fail to segregate prior to a critical period for learning (Freeberg et al. 1995). Therefore, I test the ‘first contact’ hypothesis, where juvenile cowbirds are predicted to avoid mis-imprinting on the host by following female cowbirds to conspecific foraging flocks (Hauber 2002). With an automated radio telemetry system, I determined the presence or absence (every 1-2 minutes) of radio-tagged juvenile cowbirds and their mothers within a nest box study site. I predicted that juvenile cowbirds would be located within the home-range of their mother during departures from the forest, and that departures of juveniles and mothers would correspond.

In Chapter 3, I examined the influence of reproductive performance information (personal or social) of past nesting attempts on the parasitism decisions of cowbirds. Because the reproductive output of cowbirds for a given host could reliably indicate future reproductive potential, I predicted that warbler output of cowbird fledglings and not warbler fledglings, would affect parasitism frequency in subsequent breeding attempts. First, I predicted that site-specific cowbird reproductive success would positively correlate with the likelihood of parasitism for nesting attempts within the given study site the following year. Next I compared nest boxes used in consecutive years and present results on the effects of site-specific reproductive success on the probability that non-parasitized nest boxes would be parasitized when used the following year. Finally, I accounted for potential environmental cues that cowbirds may use to select hosts, such as predation risk or food availability, with egg removal experiments. I predicted that cowbird egg removal would decrease the probability of parasitism for subsequent breeding attempts within the same nest box (both within-year and between years).

In Chapter 4, I examined the effect of brood parasitism on the likelihood of female warblers to initiate an additional brood following a successful first brood (i.e. double-brooding). Experimental manipulations have demonstrated that some songbirds respond to reductions in fecundity by increasing the frequency of double-brooding (e.g. Parejo and Danchin 2006). Consequently, avian brood parasites could induce a compensatory response (i.e. fecundity reduction) and subsequently gain additional parasitism opportunities from double-brooding. First, I predicted that the probability of double-brooding for female warblers would increase in response to natural levels of fecundity loss (i.e. compensate) from hatching failure and nestling death. Then, I compared the probability of double-brooding between parasitized and non-parasitized nests, predicting that fecundity loss associated with brood parasitism would mimic what induces female warblers to double-brood. To eliminate the possibility that female cowbirds merely parasitize warblers with an increased propensity to double-brood, I compared the double-brooding frequency among experimentally parasitized and non-parasitized nests. Because an increase in double-brooding for nests that fledged a cowbird could be a result of cowbird mortality, a potentially maladaptive association, I also used radio-telemetry to compare the double-brooding

frequency for female warblers where the cowbird juvenile perished post-fledging versus survived to independence.

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CHAPTER 2

OUT ON THEIR OWN: SOLITARY ROOSTING SUPPORTS INDEPENDENCE FROM THE HOST PARENTS IN JUVENILE PARASITIC BROWN-HEADED COWBIRDS

INTRODUCTION

Interspecific avian brood parasites exploit the parental care of host species, avoiding the energetic demands of raising offspring by laying their eggs in host species' nests. Obligate brood parasites have served as a 'model system' influencing our understanding of both the coevolution (Rothstein 1990; Feeney et al. 2014; Soler 2014) and recognition systems (Göth and Hauber 2004) of animals. Similarly, with opportunities to readily quantify the costs and benefits of this extreme form of reproduction, avian host-parasite systems also provide an opportunity to understand some constraints on the evolution of life-history strategies (Kruger 2007).

Obligate avian brood parasitism has proven to be a persistent reproductive strategy, having evolved independently at least seven times from non-parasitic, parental ancestors (Rothstein 1990; Yom-Tov and Geffen 2006). Yet brood parasitism is rare (ca. 1 % of all bird species) (Payne 1977), likely reflecting the risks arising during the initial development of interspecific brood parasitic behaviors. In nearly every test where the offspring of two bird parental species are switched early in the nestling phase, cross-fostered juveniles learn the behaviors and mate-choice decisions of their foster species (i.e. sexual imprinting; ten Cate and Vos 1999; including in facultative interspecific parasites: Sorenson et al. 2010). While sexual imprinting has significant implications for speciation by influencing the process of the recognition of suitable mates in many sexually reproducing species (Laland 1994; Irwin and Price 1999), including African brood parasites (Payne et al. 2000), sexual imprinting is conversely one of the greatest constraints on the evolution of brood parasitism (Davies 2000; Slagsvold and Hansen 2001; Sorenson et al. 2010). This leads to the question of how juvenile brood parasites avoid sexually mis-imprinting on their host species (Hauber et al. 2001).

Hybridization is rarely documented within the ~100 species of extant obligate brood parasites (e.g. Payne and Sorenson 2004); therefore, interspecific brood parasites must be capable of avoiding imprinting on their hosts. While genetically based preferences for conspecific phenotypes likely guide species recognition, learning strongly contributes to recognition systems in both parasitic and non-parasitic bird species (Payne et al. 2000; Hauber et al. 2001; Price 2008). When raised in isolation, the obligate brood parasite brown-headed cowbird (*Molothrus ater*) will respond with copulatory displays to conspecifics songs (King and West 1977) and even as nestlings, will increase begging in response to conspecific calls (Hauber et al. 2001), indicating the earliest onset of conspecific recognition capabilities. Yet by experimentally extending social contact with heterospecific species in aviaries, both cowbirds and Redheads (*Aythya Americana*; a facultative interspecific parasitic duck), preferred to sexually display to their hosts over conspecifics (Freeberg et al. 1995; Sorenson et al. 2010). Thus, laboratory experimentation has revealed a species recognition paradox, whereby brood parasites incorporate learned phenotypic components for conspecific recognition and mate choice, in spite of being raised by inappropriate referents (i.e. their host species) (Göth and Hauber 2004). By spatially segregating from the host prior to critical periods for song learning and mate choice, typical in the development of young songbirds (Bateson 1979; Hensch 2004; Brainard and Doupe 2002), juvenile brood parasites could avoid sexually imprinting on their host. Here we set out to test this hypothesis of early spatial segregation from the host by juveniles of obligate brood parasites.

Adult brood parasites are generally thought to relinquish all forms of parental care after laying their eggs, but adult parasites could influence the dispersal of their offspring away from their hosts ('first contact' hypothesis; Hauber 2002). The guidance of juvenile brood parasites away from hosts would not only reduce the chance of mis-imprinting, but the interaction with the adult would directly provide salient conspecific songs and behaviors suitable for appropriate sexual imprinting and survival. Female-assisted dispersal of offspring provides a mechanism to explain why Hahn and Fleischer (1995) reported 36% of female-juvenile cowbird pairs captured while feeding together were closely related. Similarly, evidence indicates that juvenile brood parasitic great spotted cuckoos (*Clamator glandarius*) potentially learn to

recognize conspecifics by imprinting on adult cuckoos that have maintained contact throughout the nestling and fledgling period (Soler and Soler 1999). Adult female brood parasites may have played an important role in the evolution of brood parasitism by facilitating post-fledging dispersal and initiating species recognition in their own offspring, thereby circumventing the constraint of sexually imprinting on the host species (Hauber and Dearborn 2003). Critically, the juvenile parasitic spatial segregation from hosts and the conspecifically assisted avoidance of sexual mis-imprinting hypotheses make specific predictions about the social developmental trajectory of brood parasitic young, which can be tested by extensively tracking the location of juvenile and adult parasites in space and time.

We employed an automated radio telemetry system (ARTS), where the study site occupancy of female cowbirds and their offspring could be estimated every 1-2 minutes, to investigate whether adult female brown-headed cowbirds facilitate the departure of juvenile cowbirds from their hosts. Adult female cowbirds approached conspecific juveniles in the laboratory and calls broadcasted within a forest (Hauber 2002) and likewise, juvenile cowbirds preferred to spatially associate with adult conspecific female calls over heterospecifics in field choice trials (Hauber et al. 2001), providing support for the hypothesis of female-assisted departure of their offspring. Adult female cowbirds are typically spatially faithful to a breeding area throughout the season (Dufty 1982; Raim 2000; Hahn et al. 1999; Rivers et al. 2012), further presenting female cowbirds with the opportunity to facilitate the necessary dispersal of juvenile cowbirds. We predicted that (i) juvenile cowbirds are located within their mother's home-range when juveniles depart from their host, and (ii) juvenile departures should coincide with the mother's movement away from the host. We further predicted that departure events of juvenile cowbirds experimentally introduced within a female's home range would correlate with the departure times of the representative female, suggestive of non-discriminatory kin bias in following behavior. We also investigated general patterns of juvenile departure events and female study site occupancy to determine if juvenile dispersal is guided by non-related females.

METHODS

Species and study system

We studied the movements of brown-headed cowbirds during four breeding seasons within a long-term (20+ years) nest box study system investigating the host-parasite interactions between prothonotary warblers (*Protonotaria citrea*) and cowbirds (e.g. Hoover 2003), located within the Cache River watershed, southern Illinois USA. The prothonotary warbler, a Neotropical migratory songbird that breeds in bottomland swamps throughout the southeastern USA, is the only species regularly parasitized by brown-headed cowbirds that will nest in artificial nest boxes (Petit 1999). Adult brown-headed cowbirds forage socially in pastures and agriculture fields and females cowbirds subsequently parasitize the songbirds within the surrounding landscape (Thompson 1994), particularly forests (Hahn and Hatfield 1995).

The warblers are able to successfully raise cowbird nestlings, and their nest boxes are commonly parasitized (~ 70%). We placed nest boxes 50-100m apart within suitable habitat on greased conduit poles, and upon hatching, surrounded the nest box with wire to eliminate the chance for nest predation. To reduce the effects of nest ectoparasites, such as bird blowfly maggots (*Protocalliphora spp.*) on nestling and fledgling survival (e.g. Streby et al. 2009), we replaced the nesting material every 3-5 days with Spanish moss after hatching. Each active nest box was checked every 1-2 days during the egg laying stage and then monitored every 3-5 days until fledging. Most cowbird chicks are raised in the absence of conspecifics (Lowther 1993), however, occasionally more than one cowbird are raised in a brood (e.g. Hoover 2003). Being raised with cowbird nestmates could theoretically impact the recognition system (Soler and Soler 1999), therefore, we limited each parasitized nest to a single cowbird nestling (labeled “natal” hereafter). We experimentally added a single cowbird nestling (labeled “transplanted” hereafter), collected from outside of the study sites > 1 km, to non-parasitized warbler nests and parasitized nests that failed to hatch cowbird offspring. In multiply-parasitized nests where > 1 cowbird nestling hatched, additional cowbird nestlings (1-4 days post-hatching) were moved to a nest box on a different study site with appropriately aged non-parasitized nests or where cowbird eggs failed to hatch.

Radio telemetry

During 2011-2014, we captured adult female cowbirds within the study sites by broadcasting cowbird calls adjacent to a mist net or by inserting trap doors within active warbler nest boxes prior to cowbird egg laying in the early morning (1 hour before sunrise); trap doors were then removed after cowbird capture and prior to warbler egg laying. Adult female cowbirds were captured during the first weeks of egg-laying, prior to the fledging of the juveniles. We attached radio-transmitters to adult female and juvenile cowbirds (transmitter mass = 1.6 and 0.9 g, respectively; < 5% body mass) within two distinct study sites (ABC and HB, separated by ~ 1 km; Fig. 2.1). Using the figure-8 harness method (Rappole and Tipton 1991), we affixed transmitters to the cowbird's lower back connected to elastic string looped around each thigh. We attached transmitters to juvenile cowbirds on the morning of post-hatch day 10, the average age of fledging (Woodward 1983). Using a handheld 3-element Yagi antennae and receiver, we searched for each cowbird within the study sites throughout the transmitter lifespan (adult transmitter = ~ 12 weeks; juvenile transmitter = 3-5 weeks; Holohil Systems Ltd., Ontario, CA; JDJC Corp., Fisher, IL, USA), or until the cowbird was not detected for 5 days. We identified locations for radio-tagged individuals within the forest by visual detection, or when not observed due to dense vegetation, we inferred the location based on the strength of the telemetry signal at several angles from the suspected location. Locations determined by radio-tracking were recorded using a handheld global positioning system receiver (GPS 3, Garmin, Olathe, KS, USA). We estimated the distance (m) each juvenile cowbird location was from their fledging nest box (Euclidean straight-line).

We estimated forest home-ranges for female cowbirds based on 100% minimum convex polygons (MCP), the smallest polygon that encompasses all recorded radio-tracked locations for each female within the forest for a given year. As opposed to home-range estimation techniques such as kernel estimators, MCPs have the tendency to overestimate home-range size (Powell 2000), because the polygon likely includes areas seldom used by the animal. However, we think that MCPs provide a reasonable estimate of the area where juveniles have the potential to encounter the female cowbird in question.

Automated radio telemetry system

To estimate the timing of adult and juvenile cowbird's occupancy and departure from the study sites, we deployed an automated radio telemetry system in 2012 (ARTS; Kays et al. 2011, Ward et al. 2013; Ward et al. 2014). We placed three towers adjacent to one study site (HB; Fig. 2.1): two tall towers (18 m) placed to the north/south borders and one short tower (5 m) positioned within the study site. Each tower holds six directional antennas spaced by 60° to give 360° coverage and an automated recording unit (ARU; JDJC Corp.), which systematically recorded the signal strength (in dBm) for individual radio frequencies programmed at ~1-2 minute intervals. To quantify study site occupancy, we derived signal strength thresholds, where an individual was considered present within the forest if the signal strength was greater than -130 dBm for any south antennas (120° and 180°) for tower 1, north antennas (0° , 60° and 300°) for tower 2 and any antenna on tower 3. This signal strength threshold was derived from comparisons of signal strength of juveniles and adults when known to occur within the forest from hand-tracked observations. This threshold was considered conservative in that relatively weak transmitter signals detected by the ARTS, either from individuals sitting on the ground or short-distance departures, would be considered present within the forest under this criterion. To account for electromagnetic noise, which can obscure occupancy data from apparent signals that appear to be from the radio transmitter, we estimated noise by measuring the signal strength between transmitter pulses and dismissed the data when noise was greater than -130 dBm.

We pooled binomial (yes/no) occupancy data into 30 minute intervals for analysis, and individuals were considered present within the forest if detected > 2 times during a given interval. We assumed the individual departed the forest if not detected in the subsequent 30 minute interval. In other words, we classified departure events when individuals transitioned from present to absent for at least 30 minutes. Intervals with < 3 observations, typically due to electromagnetic noise from lighting, were removed from the analysis. For analysis and presentation, we grouped time observations into distinct groups: sunrise = 1.5 hour buffer around sunrise (4:00-7:00); morning = 7:00-12:00; afternoon = 12:00-18:30; sunset = 1.5 hour buffer around sunset (18:30-21:30); night = 21:30-4:00. To determine the

reliability of ARU-detected occurrence and departure events, we compared the presence/absence data with known occurrences within the forest determined by hand-tracking.

Maternity analysis

To determine maternity of natal radio-tracked juveniles, we collected blood samples (~ 50 μ L) from the brachial vein of juvenile and adult cowbirds, both males and females captured within the forest and at a feeder trap (see below), and stored in lysis buffer at ambient temperatures or at 4⁰C. We used DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) to extract DNA from all blood samples. We used nine microsatellite primer pairs for genotyping and followed PCR amplification described in previous studies: three loci (CB 1, CB 12, and CB 15) described in Longmire et al. (2001), three loci (Ma μ 10, Ma μ 25, and Ma μ 29) described in Alderson et al. (1999), two loci (Ma μ 101 and Ma μ 104) described in Strausberger and Ashley (2001), and one (Ma μ 102) described in Strausberger and Ashley (2003). The forward primer for each locus was fluorescently labeled and analyzed on an AB 3730xl DNA analyzer to determine DNA sizes. Genotypes were assigned, both manually and automatically, using GeneMapper 3.7 (Applied Biosystems, Foster City, CA).

Deviations from Hardy-Weinberg equilibrium (HWE) of the 9 microsatellite loci were tested using Genepop'007 (Rousset 2008). For maternity analysis, we used a likelihood-based approach implemented in CERVUS 3.0 (Kalinowski et al. 2007) to assign genotyped mothers to their putative offspring. Because parentage assignment from program CERVUS depends largely on the genetic variability of the loci used and their resulting power to exclude potential parents, we attempted to increase the accuracy of allele frequencies for the study population by including the genotypes of male and female cowbirds captured at a feeder trap in addition to the adults captured within the study sites. The fly-in trap, located ~ 1 km from the study sites, was baited with a corn-sunflower seed mixture and watched continually during trapping attempts (Fig. 2.1). To determine the statistical confidence of maternity assignments, we performed a simulation of 10,000 tests based on observed genotype frequencies, assuming 90% of candidate mothers were sampled, a breeding population of 20 adult females among the

study sites and 0.01 error rate for all loci. Mother identity to radio-tagged juveniles was assigned with \geq 95% confidence, as determined by the likelihood-odds ratios (Kalinowski et al. 2007).

Statistical analyses

We used a linear mixed-effect model (LMM) to determine if the distance (m; response variable) from the natal or transplanted box was influenced by post-fledging age. To determine if the duration of time spent out of the forest after a departure event varies throughout the day, we used a LMM and included time intervals (sunrise, morning, afternoon, sunset and night) as an explanatory variable and the time after departure as the response variable. Because the duration of time and distance tended to be right-skewed, we used an exponential response distribution; results were qualitatively similar when compared to log-transformed response variables. For binary response variables, we used generalized linear mixed models (GLMM) with a binomial response, logit link function, and Laplace likelihood approximation (Bolker et al., 2009). We included whether the juvenile hand-tracked location was inside the respective female's home range (binomial response variable) with post-fledging age as an explanatory variable. To analyze the probability of juvenile departure (binomial response) we included time intervals. Similarly, we included time intervals as an explanatory variable to predict the probability of forest occupancy for adult females (binomial response). We included only those juveniles that were continually located for more than 10 days post-fledging, assuming that cowbirds disperse from their natal site 20-30 days after fledging (Woodward 1983). Juvenile models included whether or not the individual was transplanted (categorical) as an explanatory variable. All models included animal identity as a random effect to account for potential issues associated with pseudoreplication. All statistical analyses were performed in SAS (SAS 9.4, Cary, NC, USA) and parameter estimates are presented with \pm 95% confidence intervals (CI) or standard errors (SE).

RESULTS

Maternity

We included the microsatellite genotypes of 102 known adults (54 females 47 males) captured within and adjacent to the nest box study sites for maximum-likelihood simulations and to estimate

confidence of maternity assignments. The allelic frequencies for each loci were in HWE (chi-squared test: $P > 0.10$), with the exception of CB 1 ($P < 0.01$). Therefore, we eliminated CB 1 from maternity assignment analyses. We included all 54 females as candidate mothers for radio-tagged juvenile cowbirds. Of the 15 radio-tagged juveniles reared in their natal nest box, we assigned 9 (60%) to a radio-tagged mother for the given year and 2 juveniles to female cowbirds captured in subsequent years.

Forest locations

We recorded locations for juveniles every 1-2 days (mean = 17 locations for each individual), to determine the effects of age on the distance juveniles travelled from their natal nest. Results from a LMM indicated that juveniles ($n = 20$) were located at greater distances from the fledging nest box with increasing post-fledging age (Fig. 2.2; LMM: $n = 345$, $\beta = 0.13 \pm 0.01$ SE, $F_{1,324} = 190.93$, $P < 0.001$) and we found no difference in mean distance between transplanted ($n = 5$) and natal ($n = 15$) juveniles ($F_{1,324} = 0.61$, $P = 0.44$).

To determine the likelihood that juveniles would come into contact with their genetic mothers, or associated female for transplanted juveniles, we calculated the MCP as an estimate of the home range for each female cowbird. Within the two study sites, we captured and radio-tagged 15 adult females, of which 3 were radio-tagged in multiple years. One juvenile cowbird was transplanted into an area lacking a radio-tagged female cowbird, and was removed from this analysis. We found that both transplanted juveniles ($n = 4$) and natal juveniles assigned to a radio-tagged mother ($n = 9$) were more likely to be located outside of the respective female's home-range with increasing post-fledging age (Fig. 2.3; GLMM: $n = 220$; $\beta = -0.16 \pm 0.03$ SE, $F_{1,206} = 30.97$, $P < 0.001$) and found no difference between the mean probability for transplanted and natal juveniles ($F_{1,206} = 0.05$, $P = 0.83$).

Forest occupancy and departure

To determine the probability of forest occupancy and identify departures from the forest, we used data collected from the ARTS during the summers of 2012-2014 within a single study site (HB: Fig. 2.1) bordered by radio-telemetry towers. The ARTS recorded the signal strength for adult female and juvenile radio frequencies on 516,315 occasions with 227,665 identified detections (> -130 dBm). Data were

binned into 30 minute intervals ($n = 23,925$) and the ARTS ‘searched’ for the given transmitter every 1-2 minutes (mean = 21.2 times per 30 minute period). By comparing the observations collected via hand-tracking adult and juvenile cowbirds ($n = 327$), only 3 observations (1%) were discordant with the occupancy data; in each case the recorded times may have conflicted between the hand-tracked and ARTS data as occupancy was detected by the ARTS ~10 minutes after the recorded hand-tracked observation. Therefore, the occupancy estimations derived from the ARTS data appear reliable.

We tracked 10 juveniles with ARTS for the 7,296 time intervals, of which, we identified juveniles to be present within the forest on 6,718 (92%) intervals. Adult females ($n = 11$) were present in the forest 40% of the 16,629 time intervals available for detection. Assuming that a lack of detection for at least 30 minutes subsequent to being detected represents a departure from the forest, we identified 58 departure events for juvenile cowbirds (range 0-16 per individual). We found that the probability of detecting at least one departure event increased with post-fledging age (Fig. 2.4; GLMM: $n = 163$; $\beta = 0.25 \pm 0.04$ SE, $F_{1,152} = 34.28$, $P < 0.001$) and we did not find a difference between transplanted and natal juveniles ($F_{1,152} = 0.17$, $P = 0.83$). We found that the probability a juvenile left the forest varied throughout the day (Fig. 2.5a; GLMM: $n = 787$, $F_{4,773} = 6.55$, $P < 0.001$). Juvenile departures were most likely to occur (0.11 ± 0.04 SE) within 1.5 hours of sunset (18:30-21:30), twice the likelihood of afternoon mean departure probability (0.05 ± 0.02 SE). The probability of occurrence within the forest for adult female cowbirds varied throughout the day (Fig. 2.5b; GLMM: $n = 1443$, $F_{4,1431} = 123.12$, $P < 0.001$). Yet, we found that the probability of occurrence for adult females to be relatively low (0.20 ± 0.04 SE) within 1.5 hours of sunset, indicating that most juvenile departure events took place when females were unlikely to be present within the forest.

The duration that juveniles were not detected within the forest varied among time periods (Fig. 6; GLMM: $n = 54$, $F_{4,43} = 19.30$, $P < 0.001$), and the average duration was the greatest following sunset departure events (500 minutes ± 116 SE). In concordance with the departure events detected by ARTS, we documented 3 radio-tagged juveniles (range 20-30 days post-fledge) departing the forest while hand tracking within 1 hour of sunset. We were able to locate the destination for 2 of these juveniles, each

roosting in grassy fields 0.1-0.5 km from the forest for a total of 7 evenings. On both occasions, we did not observe any warbler host or other cowbirds in proximity to the recently departed juveniles. All 3 juveniles were observed within the forest the following morning, returning near where they left from and receiving parental care from their host. We located two additional radio-tagged juveniles ~ 1 km outside of the HB study site during the afternoon, and each subsequently returned to their host parents within the forest; although we were unable to determine if any cowbirds accompanied the two juveniles during their departures.

Temporal correlation in departure events

Of the 58 juvenile departure events identified by ARTS, 35 (70%) occurred on days when the assigned radio-tagged mother, or associated female for transplanted juveniles, was detected within the forest. However, we only detected 4 (6.8%) juvenile departure events occurring during the same 30 minute interval as when an adult female cowbird was detected departing the forest. We detected 1 juvenile departure event that overlapped with the departure of the genetically assigned mother, yet there were 24 minutes separating the specific departure times as determined by raw (i.e. not binned into 30 minute periods) ARTS data. The three additional temporally correlated departure events were those of transplanted juveniles, each coinciding with the departure of radio-tagged female cowbirds whose home-range did not include the respective transplanted juvenile. Additionally, we identified a single pair of juveniles that departed during the same time interval, each located outside of their respective female's home-range and located within the forest on the following morning.

DISCUSSION

Contrary to our predictions, we found no support for facilitation by adult female cowbirds being the primary way juvenile cowbirds disperse from their hosts. Juvenile cowbirds typically are not located within their mother's home range when departing the forest and likewise, we detected only one brief (~30 min.) juvenile departure event to temporally overlap with the departure of the genetically assigned mother. As observed in some cowbird populations (e.g. Hahn and Fleischer 1995, Hauber et al. 2001, Hauber 2002), female cowbirds in this study were found inhabiting the forest during the post-fledging

period of juvenile cowbirds, thus providing the opportunity for juveniles to follow female cowbirds out of the natal habitat to social foraging flocks. Critically, we found no statistical differences between natal and transplanted juveniles' and the local adult females' behaviors in this study, implying a lack of direct kin recognition in parasitic cowbird mothers. The ARTS detection analyses identified only 4 (out of 58 total) temporally correlated departures among juveniles and any radio-tagged adult female. Although, an unknown proportion of adult females inhabiting the forest were not radio-tagged, the ARTS system monitored the occupancy of 11 females and 60% of radio-tagged juveniles were genetically assigned to a radio-tagged female cowbird, indicating that a substantial proportion of females inhabiting the forest were radio-tagged. In some instances, juvenile cowbirds may have followed females without radio-tags, but we think that this is an unlikely explanation for the lack of juvenile departures coinciding with female cowbirds observed in this study. Alternatively, we detected multiple departure events from the forest for most juvenile cowbirds, increasing in probability and duration with age. In particular, we found the greatest probability for juvenile departure near sunset (18:30-21:30), when adult female cowbirds (whether radioed or not) are unlikely to occur within the forest. Visual observations of juvenile cowbirds departing the forest and subsequently roosting solitarily within grasslands provided support for the departure pattern identified by the ARTS data.

Post-fledging excursions at sunset and subsequent roosting may initiate independence from the host, thereby minimizing the possibility of sexually imprinting on the host species' phenotype. Solitary roosting has been demonstrated to increase with post-fledging age in a few non-parasitic species, such as the mourning dove (*Zenaida macroura*; Hitchcock and Mirarchi 1984) and eastern screech-owl (*Megascops asio*; Belthoff and Ritchison 1990), revealing its potential importance in general for achieving independence from parental care and initiating natal dispersal. Juvenile birds are not fed during the night, which decreases their reliance on the natal habitat and (foster) parents, and therefore departing at sunset may represent an optimal time to initiate exploratory excursions. Our study suggests support for a little explored alternative of maternally mediated conspecific recognition mechanisms in brood parasites: namely a genetic predisposition for specific roosting habitats that could induce juvenile

cowbirds to depart from the natal habitat, thus initiating the process of independence from the foster parents.

In addition to promoting spatial segregation from the host, juvenile excursions outside of the forest at sunset may also provide opportunities to locate conspecifics. Experimental manipulations of captive cowbirds have demonstrated that the production and recognition of cowbird song, and the development of appropriate social behaviors, are considerably experience-dependent (King and West 1983; O’Loughlen and Rothstein 2010; West and King 1988). Thus, the rapid discovery and joining of conspecific foraging flocks is likely critical for appropriate development. Adult cowbirds routinely fly to communal roosts in groups during the evening (Thompson 1994; pers. obs.), and juvenile cowbirds departing the forest prior to sunset would have the opportunity to locate conspecifics and potentially accompany adult cowbirds to a conspecific aggregation (i.e. communal roosts). Within the breeding season, brood parasitic Viduidae (Payne 2010) and *Molothrus* cowbirds (Ortega 1998) routinely roost with conspecifics, potentially revealing the importance of communal roosting to the evolution of brood parasitism. Therefore, roosting communally may not only enable individuals to find suitable foraging locations—‘information-center hypothesis’ (Ward and Zahavi 1973; Weatherhead 1983)—but may also enable juveniles to adopt the songs and behaviors of conspecifics. Although we were unable to hand-track any radio-tagged juvenile cowbirds to communal roosts, numerous adult females were observed associating with juveniles (all without radio-tags) at a communal roost during July 2014 (pers. obs.), indicating that departing at sunset may play a role in the juvenile cowbird’s ability to locate conspecifics.

Post-fledging birds typically disperse to specific habitat types, often dissimilar to breeding locations of the adults (Cox et al. 2014). Post-fledging habitats therefore, could promote the prevalence of conspecific interactions if habitat selection is largely innate (e.g. Partridge 1974; Grosch 2004), and shared among juvenile cowbirds and adults. Upon interaction with conspecifics within this habitat (e.g. grass pasture), genetically guided preferences for conspecific vocalizations (i.e. “passwords”; Hauber et al. 2001) and learned components such as self-referent phenotype matching (Hauber et al. 2000) would facilitate conspecific recognition and subsequently enable the acquisition of appropriate behaviors.

Spatial segregation from hosts may be an important process in the development of juvenile brood parasites and the origin of brood parasitism. As in most cross-fostering experiments (ten Cate and Vos 1999), captive juvenile brood parasites will sexually imprint on their host's phenotype when the associations are extended beyond the typical timeframe observed in nature (Freeberg et al. 1995; Sorenson et al. 2010). The redhead, an interspecific parasitic duck, primarily migrates to different wintering habitats than its host, which may enable parasitic offspring to avoid imprinting or reverse preferences acquired from interactions with the host (Sorenson et al. 2010). In support of this hypothesis, the lack of sexual imprinting observed in non-parasitic, but experimentally cross-fostered pied flycatchers (*Ficedula hypoleuca*), a non-parasitic songbird, may reflect the flycatcher's migratory behavior and resultant limitation of social interactions with experimental foster species (Slagsvold et al. 2002). While segregation behaviors of juvenile brood parasites may reduce the likelihood of mis-imprinting on host's phenotype, it does not explain how juvenile brood parasites locate and recognize conspecifics. Genetically-inherited predispositions for habitat and phenotypical characteristics shared among conspecifics, in combination with cues learned from social interactions and self-inspection, are all likely involved with obligate parasite's species recognition ontogeny (Göth and Hauber 2004; this study). Furthermore, selection has likely favored other mechanisms, such as delayed onsets of, or password-triggered flexibility in, the sensitive periods of brood parasite offspring and future research investigating these factors will help increase our understanding of the origins and evolution of brood parasitism and its complex impact on social recognition and ontogeny.

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FIGURES



Figure 2.1

The study system depicting the locations of each study site (ABC and HB), the automated radio telemetry system (ARTS) towers (stars), the baited fly-in trap (denoted by an X), and the two nest box study sites (gray polygons). The study site HB is within the ARTS coverage.

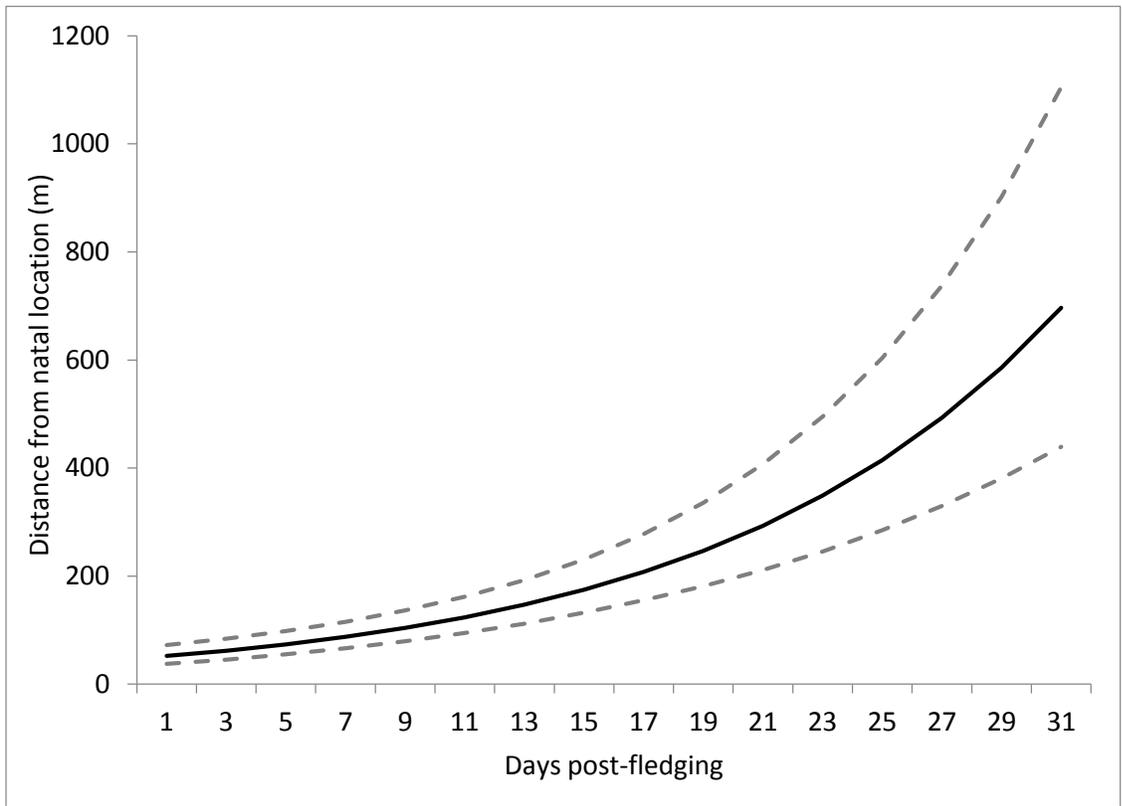


Figure 2.2

The relationship between the distance of radio-tracked locations from the natal nest box and the post-fledging age of juvenile cowbirds ($n = 20$). Mean predicted distance ($\pm 95\%$ C.I.) for a given post-fledging age shown from results of a LMM ($n = 345$ locations) with an exponential response distribution; individual identity included as a random effect.

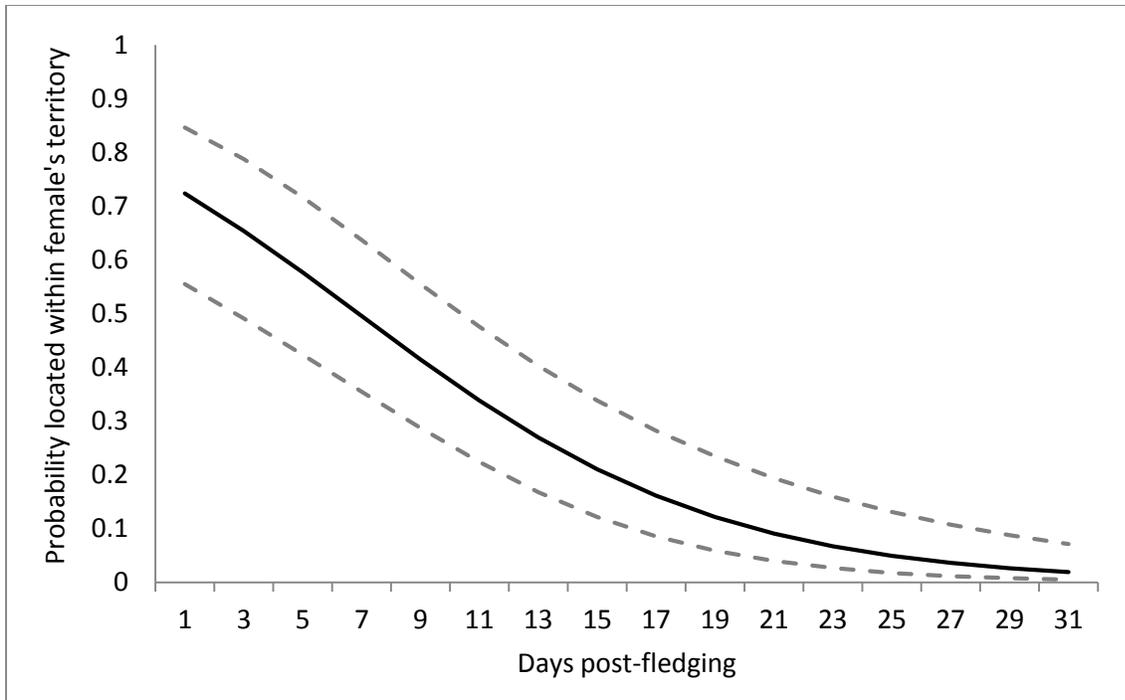


Figure 2.3

A comparison between the probability to be located within the maternal, or transplanted, female cowbird's home range and the post-fledging age of radio-tagged juvenile cowbirds (n=9). Predicted probabilities presented (\pm 95% C.I.) from a GLMM (n = 220 juvenile locations), with individual identity included as a random effect.

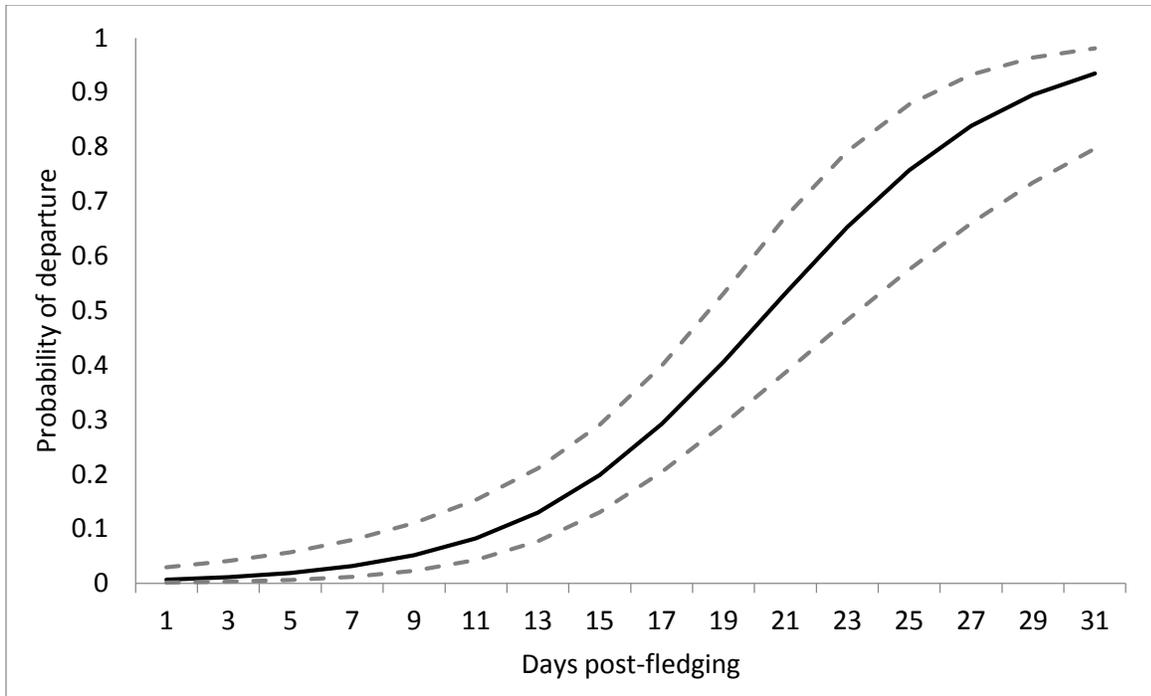


Figure 2.4

A comparison of the probability of departure (detected by ARTS) and the post-fledging age of radio-tagged juvenile cowbirds (n=10). Predicted probabilities presented (\pm 95% C.I.) from a GLMM (n = 163 observation days), with individual identity included as a random effect.

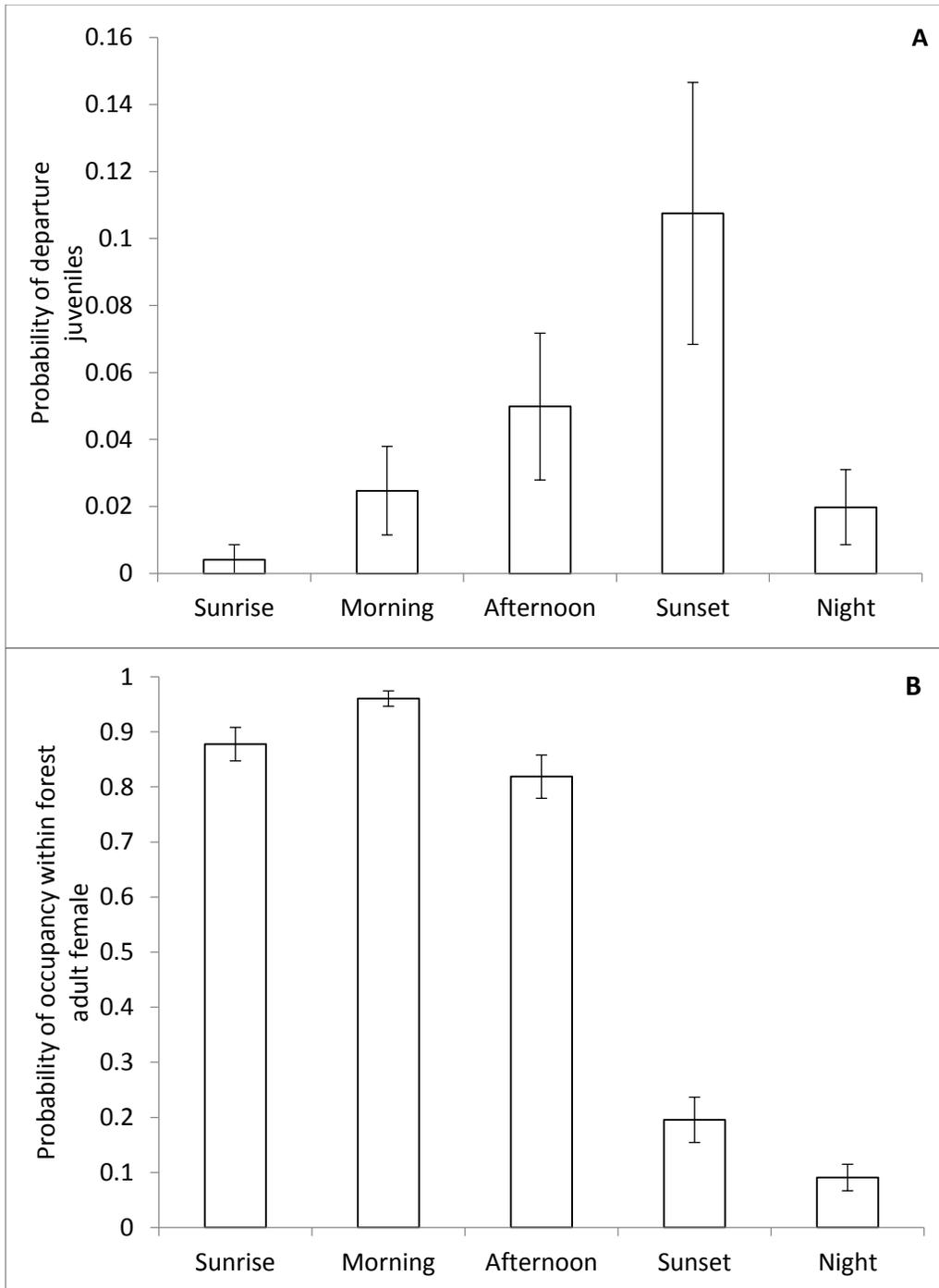


Figure 2.5
 A comparison between the (A) probability of forest departure for juvenile cowbirds (n=787 observations) and, (B) the probability of forest occupancy for 8 adult female cowbirds (n=1443 observations) in relation to time intervals. Predicted probabilities (\pm SE) are presented from results of GLMMs while including identity as a random effect.

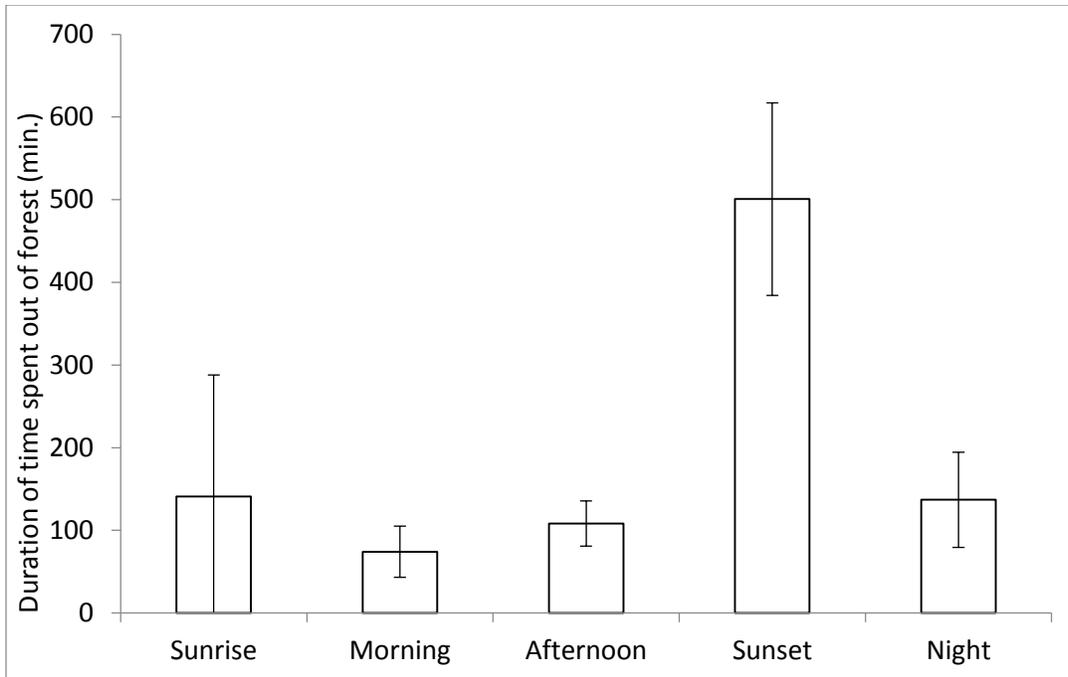


Figure 2.6
A comparison between the duration of absence during excursions outside of the forest for juvenile cowbirds (n=10) and time of day. Mean predicted duration (minutes \pm SE) for a given time interval presented from results of a LMM (n = 54 departures) with an exponential response distribution; individual identity included as a random effect.

CHAPTER 3

A GENERALIST BROOD PARASITE MODIFIES USE OF A HOST IN RESPONSE TO REPRODUCTIVE SUCCESS

INTRODUCTION

Obligate avian brood parasites, species that lay their eggs in the nests of heterospecifics, serve as a valuable resource to investigate host-parasite dynamics and coevolutionary processes (Rothstein 1990; Soler 2014; Feeney et al. 2014). Likewise, from parasitic species that choose a single host species to extreme host generalists, the range in host selectivity among avian brood parasites has intrigued evolutionary biologists (Davies 2000). Parasites are often faced with variation in the fitness potential of hosts, both among host species and within a single host across the landscape (reviewed in Soler 2014). To maximize fitness, brood parasitic females should prefer the host(s) best at raising parasitic offspring (Kleven et al., 1999; Soler et al. 1999; De Marsico and Reboresda, 2008; Grim et al. 2011) and factor in host defenses against parasitism, availability and compatibility when choosing a host. As host defenses evolve, coevolutionary theory predicts that avian brood parasites increase in host specificity (Davies and Brooke 1989; Rothstein et al. 2002). The brown-headed cowbird (*Molothrus ater*), is an extreme host generalist known to parasitize more than 220 species throughout its range (Lowther 1993; Ortega 1998). The degree to which female cowbirds select hosts based on the variation in fitness potential is poorly known (Briskie et al. 1990; Curson et al. 2010). While *hosts* have been observed to modify their behavior in response to past interactions with brood parasites (Langmore et al. 2012; Feeney and Langmore 2013; Molina-Morales et al. 2014), adaptive host choice by obligate interspecific parasites in response to previous reproductive success has not been demonstrated.

The quality of potential hosts of brown-headed cowbirds (cowbirds hereafter) is highly variable, but potentially predictable and depends on the likelihood that a particular host species can recognize and reject parasitic eggs (Rothstein 1990) or is in some way incompatible with raising a parasitic egg or chick (Middleton 1977). For example, of the ~220 parasitized species, approximately 140 have successfully

raised a cowbird offspring (Lowther 1993). Interspecific brood parasites could enhance reproductive success by preferring species, locations or individuals that fledged offspring (host or parasitic) in the recent past (e.g. Pöysä 2003; Pöysä 2006). Similarly, host success could be an indirect cue of host quality in systems where cowbird success is principally dependent on nest predation (Avilés et al. 2006). When reproductive success is predictable, reproductive performance is among the most reliable sources of biological information, enabling individuals to enhance fitness by minimizing uncertainty associated with breeding in heterogeneous environments (Danchin et al. 2004; Schmidt et al. 2010). Female brown-headed cowbirds, faced with variation in the fitness potential among reproductive decisions, could draw upon the outcomes of past breeding experiences or observations of conspecifics and heterospecifics to maximize fitness within a given environment (i.e. use personal experience or social information).

Despite host generalism observed at the species-level, microsatellite DNA markers revealed evidence of host preferences among individual female cowbirds in several populations (Alderson et al. 1999; Hahn et al. 1999; Woolfenden et al. 2004; Strausberger and Ashley 2005). Egg laying decisions of inexperienced female cowbirds, either young individuals or those recently dispersed to novel habitats, may follow a fairly indiscriminate pattern (McLaren et al. 2003). If the success of parasitized nests is monitored, subsequent decisions to use particular hosts could follow statistical decision theory where prior preferences are refined to match collected information sampled from the environment (Dall et al. 2005). Radio telemetry and genetic studies of female cowbirds also indicate high breeding site and home range (primary egg-laying area) fidelity between years (Dufty 1982; Hahn et al. 1999) which would allow females to gather information and use it in subsequent breeding decisions. Cowbird females are equipped with impressive spatial memories (Sherry et al. 1993; Guigeno et al. 2014) and also monitor host nest contents both before and after parasitism, to time the laying of eggs and ensure their acceptance by the host (Hoover and Robinson 2007). Parasitized nest sites are often parasitized again on subsequent attempts (Hauber et al. 2004; Hoover et al. 2006), potentially indicating preferences for particular individual hosts. Evidence of preferences for particular hosts, in combination with host nest monitoring and site fidelity within cowbird populations, suggests cowbirds could improve future breeding decisions

by incorporating their own reproductive experience or the reproductive success of conspecifics into parasitism decisions.

Variation in anti-parasite strategies, microhabitat nesting structure, timing of breeding and abundance among the potential host species likely contributes to the egg laying decisions of generalist brood parasites. Studying the parasitism frequencies within a single host. Therefore, would control for the between-species variation that may obscure the patterns of parasites using past reproductive performance to make egg laying decisions. Studies investigating the parasitism frequencies within a single host have revealed increased parasitism rates for host traits (e.g. nest quality, body condition and social dominance) that may be correlated with the host's ability to successfully raise parasitic offspring (reviewed in Parejo & Avilés 2007; Feeney et al. 2012). However, it remains unclear whether the past reproductive experiences of individual brood parasites, rather than fixed preferences for the host traits themselves, serve as a cue that influences host choice within interspecific brood parasites.

In this study, we used a long-term (21 years) nest box dataset from a highly suitable brown-headed cowbird host, the prothonotary warbler (*Protonotaria citrea*; warbler hereafter), to investigate the factors influencing cowbird parasitism. Here, we test if the warblers' ability to successfully produce warbler and/or cowbird offspring influence(s) the probability of cowbird parasitism in subsequent breeding attempts, both within the same season and between consecutive years. From a cowbird's perspective, the fledging of cowbirds is likely a better signal of host quality than the fledging of host offspring; therefore, we hypothesized that parasitism frequency would increase in response to cowbird fledging success rather than warbler fledging success. First, we predicted that as the number of cowbirds fledging per warbler nest (i.e. cowbird productivity) on a site increased, the following year's probability of cowbird parasitism for warblers on that site would increase, whereas the number of warblers fledging per nesting attempt on a site would have relatively little influence on the rate of future cowbird parasitism. Next, we investigated the influence of cowbird productivity on the parasitism status of individual nest boxes used in consecutive years. If the reproductive performance information beyond a specific nest box is used to influence the parasitism decisions of female cowbirds, we would predict that study-site-specific

cowbird success would positively correlate with the probability that non-parasitized nest boxes would become parasitized the following year rather than just previously parasitized boxes continually being parasitized. Finally, by directly manipulating the reproductive success of cowbirds via experimental egg removal, we accounted for potential confounds of environmental factors that cowbirds may use as predictors of reproductive success, such as predation risk or food abundance. Compared to parasitized nests that successfully fledged cowbird offspring, we predicted that experimentally removing cowbird eggs would decrease the probability of parasitism for the subsequent nesting attempts, both between years and between multiple nesting attempts within the same year.

METHODS

Study species and field methods

The study was conducted over a 21-year period (1994 to 2014) in the Cache River Watershed in southern Illinois, United States (37°18'N, 88°58'W). Here the prothonotary warbler, a territorial and socially monogamous Neotropical migratory songbird (Petit 1999) that lacks any known anti-parasite defenses (Hoover 2003b), uses nest boxes that provide easy access for monitoring parasitized nests. Study sites were located in agriculturally fragmented patches of forested sloughs and floodplains with bald cypress (*Taxodium distichum*) and tupelo (*Nyssa aquatica*) swamps, within a 192-km² portion of the watershed. Warbler young are fed an insectivorous diet by both parents that is compatible with the diet required to raise cowbird young (Hoover and Reetz 2006). Cowbird young are competitive for provisioned food because they are larger than warbler young throughout the nestling and fledgling stage. Despite a high rate of cowbird parasitism (~60% of nests) and their larger size, the warblers are often double-brooded and capable of raising both cowbird and host nestlings in each nesting attempt (Hoover 2003c; Louder et al. 2015).

Each year we set up and monitored approximately 1000 warbler nest boxes across 21 sites (individual patches of suitable breeding habitat for warblers separated by more than 1 km of non-suitable habitat). Nest boxes were made from modified 1.9 L beverage cartons and placed on trees about 1.7 m above the ground in suitable habitat. Nest boxes were spaced an average of 50 m apart, and openings in

boxes were made to be the average diameter (44 mm) of warbler nests in natural cavities allowing cowbird access to each nest. Study sites where the opening size of nest boxes was reduced to exclude parasitism by cowbirds were not included in our analyses. We monitored boxes every 3-5 days from late April to early August. The status of each nesting attempt was recorded, including the number of warbler and cowbird eggs, nestlings, and the number of cowbird eggs that were removed. We considered nestlings to have fledged if they reached 10-11 days of age and the nest was empty and intact on the subsequent visit. Additional evidence of fledging included the presence of trampled droppings in the nest, alarm calls from adults, and observations of appropriately-aged fledglings in the territory. Adult warblers were captured and fitted with a unique color-band combination and a numbered aluminum band. The identities of ca. 90% of the adults from each nesting attempt were recorded by either capture or visual identification.

Study-site-specific cowbird reproductive success

To ensure that our egg removal manipulations did not account for correlations among reproductive success and parasitism likelihood, we first investigated a nest box dataset including study sites/years where cowbird eggs were not removed. To test whether the site-specific production of warbler or cowbird offspring in one year (t) best predicted site-specific cowbird parasitism in the following year ($t+1$), we included site-specific warbler reproductive success for year (t) (number of warblers fledged/number of warbler nesting attempts/site), and site-specific cowbird reproductive success for year (t) (number of cowbirds fledged/number of warbler nesting attempts/site) as potential explanatory variables. We controlled for the seasonal decline in cowbird parasitism by including month of the nesting attempt (April-July) as a continuous covariate in year ($t+1$). In order to control for landscape-level effects of cowbird parasitism in our analysis, where the site-specific parasitism rate was correlated among years because the configuration of forest habitat and cowbird foraging areas remained nearly constant (Goguen and Matthews 2000; Hoover and Hauber 2007), we included the ambient parasitism rate from the respective study site from the year (t) (number of warbler nests parasitized/number of warbler nesting attempts per site). Some studies have found variation in parasitism with respect to host density (e.g.

Barber and Martin 1997; Woolfenden et al. 2004; Stokke et al. 2007), therefore we included the site-specific warbler density (number of females/hectare) to investigate if a current condition such as the density of a quality host predicts the probability of cowbird parasitism in year (t+1).

Next, to elucidate whether site-wide cowbird success in year (t) positively correlates with the probability of parasitism in both parasitized and non-parasitized nest boxes in year (t+1), indicative of reproductive performance information used beyond a specific nest box, we used a subset of data that included individual nest boxes used by warblers in consecutive years from non-manipulated (i.e. without egg removal) study sites. We included nesting attempts initiated in May of each year to control for the seasonal decline in parasitism rates and matched individual nest boxes that were used in two consecutive years. We included the parasitism status (categorical) and site-wide cowbird success (continuous) in year (t) as explanatory variables and an interaction between the variables to predict the parasitism probability for the same individual nest box in the following year (t+1).

Experimental cowbird egg removal

We experimentally manipulated cowbird reproductive success with predator exclusion and cowbird egg removal to reduce the potential confounds of environmental (e.g. water depth; Hoover 2006) and host characteristics that may affect cowbird reproductive success and thus, potentially serve as cues for cowbird egg-laying decisions. We placed nest boxes on two conduit poles with axle grease at least one meter from the closest vegetation to eliminate nest predation and thus, maximize the likelihood of cowbirds fledging from a parasitized nest. In approximately two thirds of study sites each year (1999-2014), we removed an estimated 20-100% of cowbird eggs laid in a given study site. Parasitized ‘removal’ nests, where all cowbird eggs were removed prior to a complete clutch or during incubation (days 1-5), were compared to parasitized ‘fledged’ nests where at least one cowbird offspring fledged in addition to warbler fledglings. The majority of ‘fledged’ nest boxes involved no cowbird egg removal and produced a single cowbird fledgling. Parasitized nest boxes which failed to fledge a cowbird due to natural hatching failure or nestling death were not included in analyses. We included the number of

cowbird eggs laid (continuous) in year (t) for each nest as a potential explanatory variable of the parasitism probability the year (t+1).

Next, we investigated the influence of cowbird and host reproductive output on the within-season parasitism probability of subsequent breeding attempts, using a dataset including only female warblers that were parasitized during their successful first nest and then initiated a subsequent attempt (i.e. double-brooding). We compared the probabilities of parasitism for second breeding attempts between females that fledged any cowbird offspring and females that fledged only warblers (where all cowbird eggs were removed) in their first attempt. In addition to the effect of egg removal (categorical yes/no), we included the number of cowbird eggs laid (categorical), number of warblers fledging (categorical), and the initiation date of the second breeding attempt (ordinal date) as potential explanatory variables.

Preference for individual warblers or nest boxes

Finally, we investigated whether there was any indication that female cowbirds focused their parasitism decisions on particular female warblers or nest boxes. Here we included only nest boxes that successfully fledged at least one cowbird and we compared the future parasitism status (categorical yes/no) among three categories of nests for both within- and between-year datasets. These categories of nests were: 1) same female warbler occupied the same nest box where she successfully fledged a cowbird in the previous attempt, 2) same female warbler occupied a different nest box than where she successfully fledged a cowbird in the previous attempt, and 3) new female in a nest box that had fledged a cowbird in the previous attempt. We performed this analysis for both within- and between-year datasets. If female cowbirds focused on particular female warblers that had successfully raised a cowbird, then categories 1 and 2 should have higher rates of parasitism than category 3. If instead female cowbirds focused on particular nest boxes that had fledged a cowbird, then categories 2 and 3 should have higher rates of parasitism than category 1. No difference in parasitism rates among categories would indicate that female cowbirds lack preferences for particular nest boxes or female warblers. To control for potential issues of non-independence, female warblers were only used once in each dataset.

Statistical analyses

We analyzed the probability of cowbird parasitism, a binary response variable, by using a series of generalized linear mixed models (GLMMs) with Laplace approximation of the log likelihood (Bolker et al. 2009) and a logit link function (GLIMMIX; SAS 9.4; SAS Institute, Inc., Cary, NC, USA). We did not include correlated explanatory variables ($r > 0.70$) in the same model to reduce any effects of collinearity among variables. We included study site as a random effect in all models and presented model parameter estimates and associated standard errors (\pm SE). We used Pearson's chi-square tests to compare the frequency of parasitism among the three categories of nests (i.e. preference for individual warblers or nest boxes).

RESULTS

Study-site-specific cowbird reproductive success

Of the 1458 warbler nests from non-manipulated study sites, 962 (66%) were parasitized by brown-headed cowbirds. As expected, the rate of parasitism in year (t) ($\beta = 2.02$; $F_{1,1440} = 12.04$; $P < 0.001$) and month ($\beta = -1.41$; $F_{1,1440} = 145.46$; $P < 0.001$) were positively correlated with probability of parasitism in the following year (t+1). The probability of parasitism in year (t+1) was positively influenced by cowbird fledging success in year (t) ($\beta = 1.54$; $F_{1,1440} = 5.62$; $P = 0.02$), increasing from 52% (± 0.09 SE) when there was no cowbird fledging success to 73% (± 0.09 SE) when cowbird fledging success was at its greatest observed levels in year (t) (0.6 cowbird fledged per warbler nesting attempt; Fig. 3.1). Surprisingly, we found a relatively small ($\beta = -0.58$) but significant negative effect of site-specific warbler reproductive output in year (t) on the probability of parasitism in year (t+1); ($F_{1,1440} = 5.62$; $P = 0.02$), where sites that produced relatively few warblers per nesting attempt exhibited an increased likelihood of parasitism the following year. We found no effect of female warbler density on the parasitism rate for the current year ($\beta = 0.11$; $F_{1,1440} = 0.64$; $P = 0.42$).

We then compared the influence of site-specific cowbird success on either parasitized or non-parasitized nest boxes used in consecutive years on non-manipulated study sites using 245 nest boxes that were initiated in May of each year. We found that parasitism status (GLMM: $F_{1,229} = 6.09$; $P = 0.01$) and

site-specific cowbird reproductive success ($\beta = 2.56$; $F_{1,229} = 4.78$; $P = 0.03$) in year (t) were positively associated with the probability of parasitism for the respective nest box when used the following year (t+1). In particular, non-parasitized nest boxes exhibited a strong increase in parasitism likelihood the following year in response to the previous year's site-specific cowbird success rate (Fig. 3.2). There was no support for an effect of the interaction between cowbird success and parasitism status on the likelihood of parasitism between years ($F_{1,229} = 1.20$; $P = 0.27$).

Experimental cowbird egg removal

Of the 355 parasitized nest boxes that successfully fledged any offspring (i.e. host or cowbird) from experimental study sites in year (t) that were used by warblers in year (t+1), we removed all cowbird eggs from 117 nests. The removal of cowbird eggs explained variation among parasitism frequencies between years (GLMM: $F_{1,334} = 4.13$; $P = 0.04$) where the probability of parasitism for nests that fledged cowbirds (0.87 ± 0.03 SE) was greater than nests where cowbird eggs were removed (0.79 ± 0.05 SE; Fig. 3.3). The number of cowbird eggs laid in year (t) was not correlated with likelihood of parasitism the following year (t+1) ($\beta = 0.19$; $F_{1,334} = 1.52$; $P = 0.22$).

For the within-season analysis of the probability of parasitism during the second brood in relation to cowbird success, we included 363 female warblers parasitized during their first broods that successfully fledged any offspring (i.e. host or cowbird) and initiated an additional attempt (i.e. double-brooding). We removed all cowbird eggs from 115 of these first broods. More than 90% of second clutches were initiated within the same or adjacent nest box as the first successful nest. In a GLMM, we controlled for the potential effects of the number of cowbird eggs laid ($\beta = 0.27$; $F_{1,326} = 4.63$; $P = 0.03$), number of warblers fledged ($\beta = 0.06$; $F_{1,326} = 0.42$; $P = 0.52$), and the initiation date of the second brood ($\beta = -0.02$; $F_{1,326} = 2.57$; $P = 0.11$) on the probability of parasitism during the second brood. The probability of parasitism for female warblers fledging ≥ 1 cowbird offspring during their first breeding attempt (0.38 ± 0.06) was twice that of nests where we experimentally removed all cowbird eggs (0.15 ± 0.05 ; Fig. 3.4).

Preference for individual warblers or nest boxes

For our between-season dataset that included successful first nests that fledged at least one cowbird we found no significant difference in parasitism rates among the three categories of nesting scenarios ($X^2 = 0.72$, $n = 915$, $d.f. = 2$, $P = 0.70$): category 1 (61%) where a female warbler that successfully fledged a cowbird in the same nest box as the previous year, category 2 (57%) where a female warbler fledged a cowbird using a different nest box than the previous year, and category 3 (59%) where a new female warbler nests in a box that had fledged a cowbird in the previous year. Therefore, we found no evidence of cowbirds preferring particular nest boxes or female warblers between years.

For our within-season dataset that included successful first nests that fledged at least one cowbird, we found that parasitism rate in the second attempt tended to be greater when the same female warbler used the same nest box as the previous attempt (Category 1 = 35%) or when a female warbler used a different nest box but still in the study site (Category 2 = 46%), when compared to a nest box that had fledged a cowbird in the previous attempt but had a new female warbler (Category 3 = 20%), however this relationship was not significant ($X^2 = 4.67$, $n = 197$, $d.f. = 2$, $P = 0.10$). Therefore, we found only marginal support for within-year host selection based on individual female warblers.

DISCUSSION

Our analyses revealed that the probability of parasitism for prothonotary warblers increased in response to brown-headed cowbird reproductive success both between breeding attempts within the same year and between breeding seasons. We identified a positive correlation for site-specific cowbird success in year (t) and the probability of parasitism the following year ($t+1$), even after controlling for confounding factors (site-specific parasitism rate and date) known to be important in our study system. In particular, we detected a strong positive correlation between the site-specific cowbird success one year and the likelihood of parasitism the following year for nest boxes that had not been parasitized the previous year. Furthermore, our experimental removal of cowbird eggs decreased the probability of parasitism for subsequent nesting attempts both within and between years. Our results indicate that host use by female cowbirds is not merely a function of landscape characteristics (e.g. fragmentation, edge

effects), but also reflects the use of reproductive performance information from past parasitism attempts. Furthermore, we know that cowbirds can discriminate between areas with high and low cowbird productivity because we saw elevated and reduced parasitism, respectively, within the same host species using uniform nest sites (i.e. nest boxes). Therefore, we conclude that cowbirds are not simply selecting hosts in our study system based on natural history characteristics (e.g. habitat, nest site).

Female cowbirds may draw upon their own breeding experience (i.e. personal information) or that of other cowbird females (i.e. social information), targeting productive locations and hosts to increase their reproductive output. The use of personal and social information in breeding decisions has been widely investigated in non-parasitic passerines (e.g. Doligez et al. 2002; Hoover 2003a; Danchin et al. 2004), but this is the first example of experiential information use documented within an obligate brood parasitic species. Lacking genetic data depicting the egg-laying decisions for individual female cowbirds, we can only infer that females use both personal and social information to enhance their reproductive potential. Because female cowbirds are often faithful to an egg-laying area (unpublished data), both within and between years, our experimental removal of cowbird eggs and resultant decrease in parasitism probability for subsequent breeding attempts within the same nest box suggests that personal information is used to inform egg-laying decisions. The likelihood of parasitism increased dramatically for non-parasitized nest boxes when used again in the year following a year with high site-wide cowbird success. This result may reflect site-faithful female cowbirds increasing egg-laying in response to their own breeding experience and other female cowbirds similarly responding to the cowbird productivity observed via prospecting. Furthermore, females could collect social information regarding the breeding habitat, nest type, or other natural history characteristics of hosts that successfully rear cowbird offspring (Mahler et al. 2007). This may lead to the immigration of adult female cowbirds into habitat patches where warblers are breeding, thereby increasing the number of cowbirds and, consequently, the probability of parasitism for warblers.

Parasitism rates have been found to be positively correlated with host characteristics, such as nest quality, body condition and social dominance (reviewed in Parejo & Avilés 2007; Feeney et al. 2012). As

many of these host characters are sexually selected traits thought to serve as cues indicative of parental abilities, brood parasites potentially choose hosts based on trait conspicuousness or by collecting inadvertent information (i.e. eavesdropping), if these host characters reflect the ability to successfully raise parasitic offspring. Experimentally removing cowbird eggs, however, resulted in decreases in parasitism probabilities on subsequent nesting attempts and morphological traits are not found to be correlated with parasitism rates for prothonotary warblers (Hoover and Hauber 2006), indicating that host traits are relatively unimportant influences on parasitism decisions within our study population. Therefore, the apparent preference for host traits observed in other studies may reflect brood parasites responding to success, rather than eavesdropping on heterospecific signals. One might expect cowbirds generally to parasitize hosts that experience low rates of nest predation, indicated by high fledging success of host young, particularly if cowbirds are able to assess that the presence of many host fledglings on a site is a function of low rates of nest predation. Conversely, our results indicate that warbler reproductive output is not a good predictor of cowbird parasitism in the subsequent nesting attempt or between breeding seasons. In our study system, outcomes of past parasitic attempts, rather than host traits or the number of warbler offspring fledged, influenced the egg laying decisions of female cowbirds; suggesting that female cowbirds have a sophisticated ability to discriminate between information sources.

If juvenile cowbirds return to their natal location and/or host species to breed in the subsequent years, local recruitment of cowbird offspring on productive sites could account for the rise in parasitism between years in response to cowbird reproductive success. Juveniles may preferentially parasitize the species that raised them by imprinting on the host species itself (Payne and Payne 1998; Payne et al. 2000), on the nest characteristics of that species (Mahler et al. 2007), or on the habitat it was raised in (Teuschl et al. 1998). As a cavity nesting passerine, the prothonotary warbler could offer cowbirds a unique nestling experience and search image compared to other available species in the host community. While many adult brood parasitic species exhibit breeding site fidelity (Dufty 1982; Soler et al. 1995; Raim 2000; Langmore et al. 2007), natal philopatry for cowbirds is considered uncommon (Hauber et al. 2012). Because cowbirds do not breed until ≥ 1 year old (Lowther 1993), the effects of egg removal on

parasitism rates between consecutive breeding attempts within a given year (i.e. double-brooding) indicates that at the very least, adult female cowbirds use reproductive performance information to guide parasitism decisions within-season. Therefore, the observed increase in the probability of parasitism between years in response to cowbird reproductive success is more likely explained by female breeding site fidelity rather than by the local recruitment of cowbird offspring alone.

Warbler density in the current year was examined as a potential predictor of cowbird parasitism to investigate if female cowbirds parasitize warblers based on their abundance (e.g. Barber and Martin 1997; Woolfenden et al. 2004), instead of their ability to fledge cowbird young. The influence of host density on patterns of parasitism may vary depending on the host specificity of the brood parasite and/or the quality of the host species parasitized (Jensen and Cully 2005). The rate of cowbird parasitism for prothonotary warblers could fluctuate in response to changes in the availability of alternative hosts, but we did not measure changes in the densities or rates of cowbird parasitism of other hosts during the course of this study. Nevertheless, it is difficult to imagine how the availability of alternative hosts in the current year could fluctuate in parallel with cowbird reproductive success in a way that would undermine the effect on cowbird parasitism of the warblers observed both within and between years.

While we were unable to determine the frequency of cowbird parasitism for alternative hosts within the community, we would predict that the use of reproductive performance information could lead to greater host specificity within individual females as they hone their ability to choose host species that are better able to fledge parasitic young during several consecutive breeding seasons. Brood parasites are able to forego much of the energetic and temporal costs of parental care to complete reproduction, yet each parasitism event is associated with alternative costs such as nest searching and egg laying while physiological (Holford and Roby 1993) and temporal constraints (Curson and Mathews 2003) limit the number of nests female cowbirds can parasitize in a given year. Therefore, increasing the parasitism frequency for one host would likely decrease the parasitism risk for other hosts within the female's egg laying range. Although parasitism frequencies do not always correspond to the host species considered most successful at producing cowbird offspring in other systems (e.g. Briskie et al. 1990; Kattan 1997;

Curson et al. 2010), variation in anti-parasite strategies, microhabitat structure and temporal host availability within the study population can complicate attempts to estimate host quality and may prohibit the detection of experiential information use. Furthermore, host quality may not be predictable for some species in heterogeneous environments, reducing the reliability of reproductive performance information in some systems. Taking into account local productivity at the scale of individual females will help elucidate this pattern in other host-parasite systems.

Our results suggest that the rate of parasitism for a high quality host was best predicted by the brown-headed cowbird reproductive success of previous breeding attempts. This pattern implies that cowbird females are monitoring the reproductive output to inform future breeding decisions. By experimentally eliminating cowbird success, we decreased the probability of parasitism for the nest box on subsequent attempts, yet we found no supporting evidence that female cowbirds preferentially parasitize particular female warblers or nest boxes that fledged a cowbird. Together, this suggests that cowbird females in our study system are not necessarily tracking individual female warblers or nest boxes, but may be tracking cowbird production for this host species at the scale of female cowbird egg-laying ranges within a study site (which encompass several warbler territories). Female cowbirds likely are using some combination of personal and social information associated with their own and neighboring female cowbird's success to modify their egg-laying decisions, both within and between breeding seasons. Future experimental research involving genetic analyses should help to elucidate the relative importance of personal and social information within female cowbird host-use decisions and whether increases in parasitism are associated with repeated parasitism from known individuals versus an influx of new or young cowbird females.

Coevolutionary theory predicts that raising the offspring of heterospecifics, at a fitness cost for the host, generally results in selective pressures favoring the development of anti-parasite strategies in brood parasites (reviewed in Feeney et al. 2014). Here we suggest that the parasite's use of reproductive performance information and the ensuing preference for successful hosts could regulate host population sizes, thus increasing the selective pressure for the development of defense strategies in hosts.

Conversely, reduced parasitism pressure for poor hosts could effectively delay the increase of host resistance when initiated within a given population, as the rate of development and spread of parasite rejection behaviors for hosts depends on the parasitism frequency (Kelly 1987; Takasu 1998). Parasite resistance appears to be largely genetically driven (Soler et al. 1999; Kuehn et al. 2014), therefore, if brood parasites avoid egg rejecters, the prevalence of brood parasite egg rejection in hosts could decline for a given host population (Marchetti 1992); analogous to host populations with increasing duration of allopatry from brood parasites and the observed decline in resistant strategies (Brooke et al. 1998). By limiting the effectiveness of host resistance, and in turn delaying the coevolutionary host-parasite arms race, parasites are theoretically more able to have a wider host range (Kawecki 1998). Further investigations into the development of preferences for hosts have the potential to enhance our understanding of the evolution of host specificity and the effect of parasite cognition on the development of anti-parasite defenses in hosts.

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FIGURES

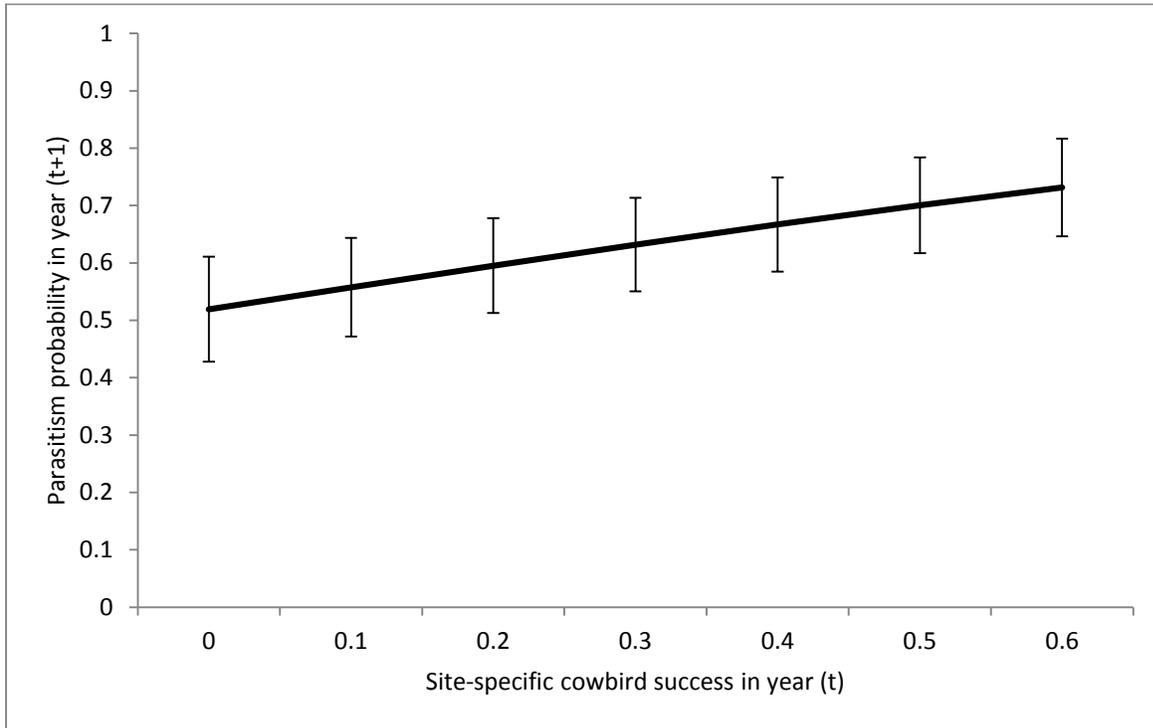


Figure 3.1

The relationship between the site-specific cowbird reproductive success in year (t) and the probability of parasitism for nests within that given study site the following year (t+1). Results of a GLMM (n = 1458 nests) and the mean predicted probability of parasitism (\pm SE) are presented while holding additional explanatory variables at mean observed values; data includes nests from non-manipulated study sites (i.e. without egg removal) and study site was included as a random effect.

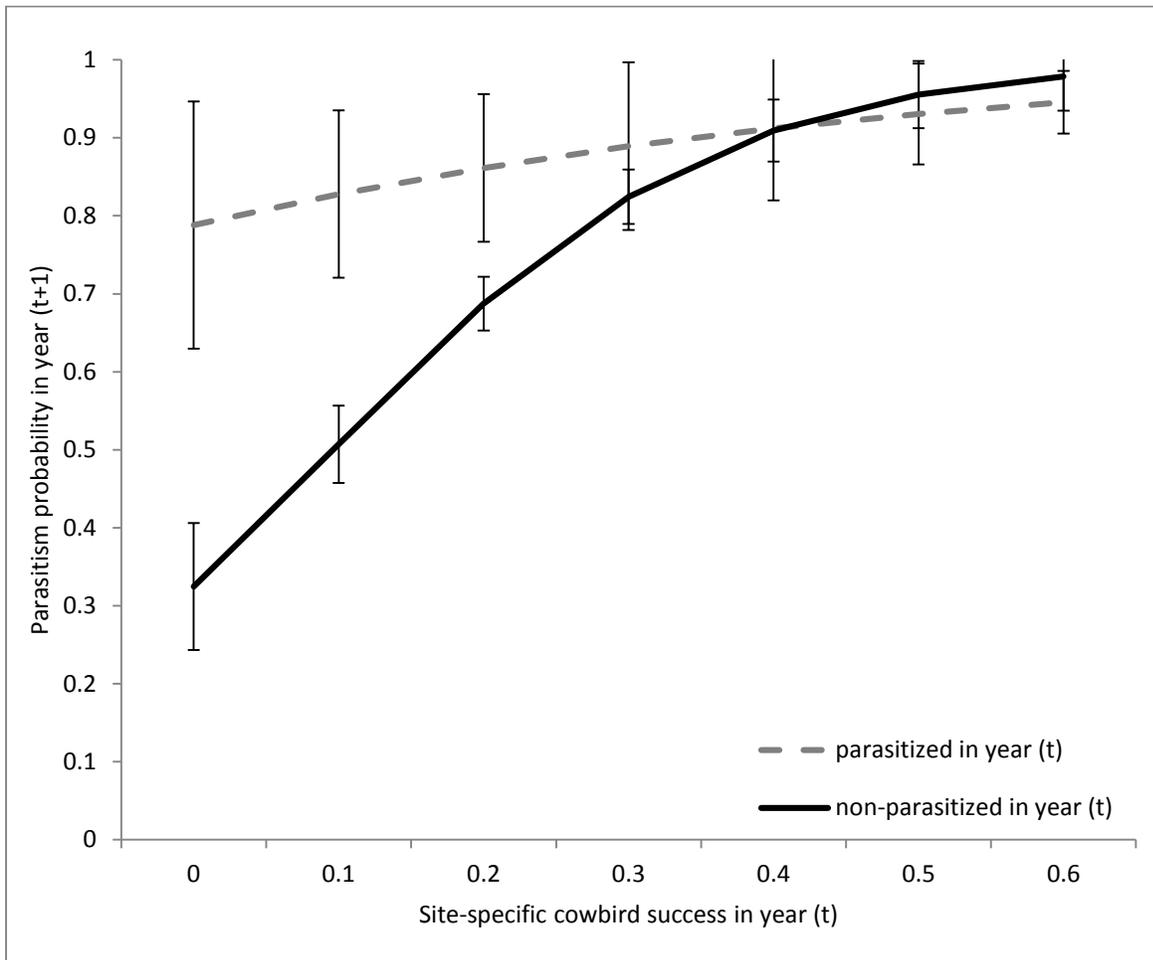


Figure 3.2

The comparison between the site-specific cowbird reproductive success in year (t) and the probability of parasitism the following year (t+1) for nest boxes used by warblers in consecutive years; nest boxes parasitized (gray dotted line) in year (t) and non-parasitized (black line) in year (t). Results of a GLMM (n = 245 nests) and the mean predicted probability of parasitism (\pm SE) are presented while holding additional explanatory variables at mean observed values; data includes nests from non-manipulated study sites (i.e. without egg removal) and study site was included as a random effect.

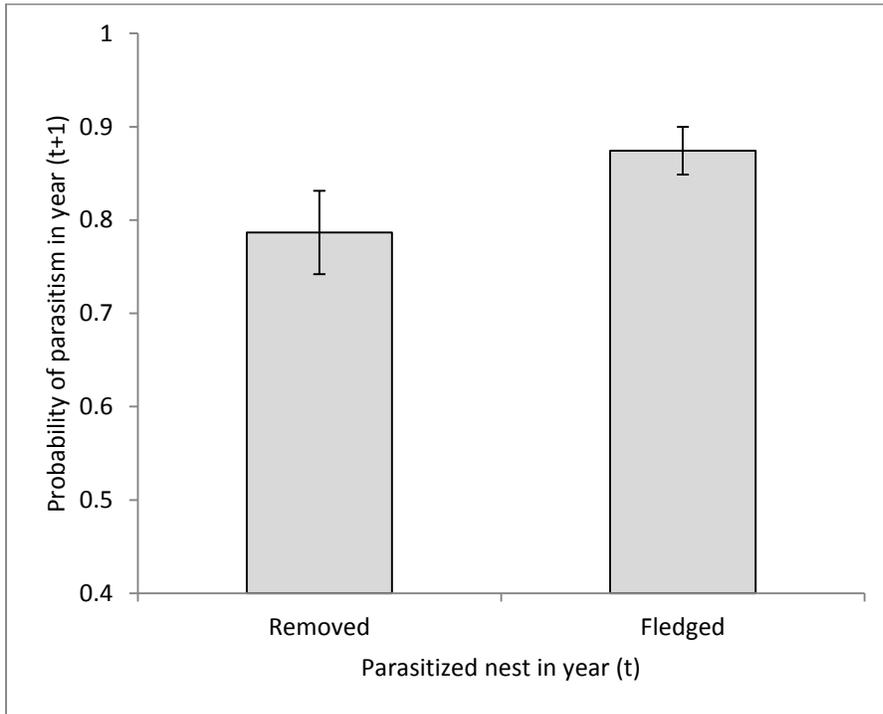


Figure 3.3

The comparison between parasitized nests where all cowbird eggs were removed versus nests where ≥ 1 cowbird offspring fledged in year (t) and the probability of parasitism the following year (t+1) for nest boxes used by warblers in consecutive years. Results of a GLMM (n = 355 nests) and the mean predicted probability of parasitism (\pm SE) are presented while holding additional explanatory variables at mean observed values; study site included as a random effect.

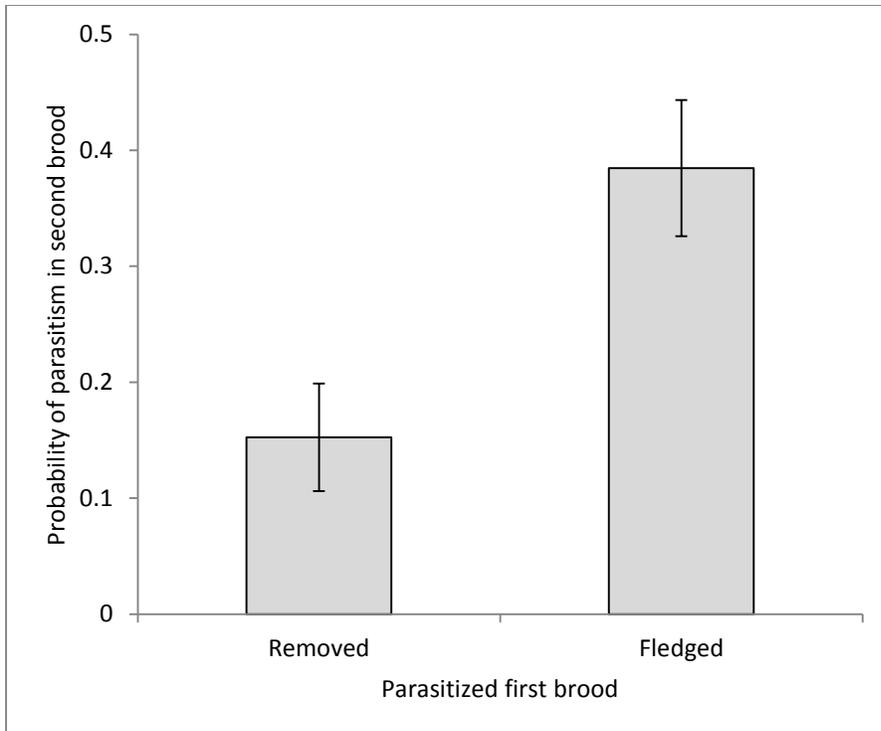


Figure 3.4

The relationship between parasitized first broods where all cowbird eggs were removed versus nests where ≥ 1 cowbird offspring fledged and the probability of parasitism for the second brood (i.e. double-brooding). Results of a GLMM ($n = 363$ nests) and the mean predicted probability of parasitism (\pm SE) are presented while holding additional explanatory variables at mean observed values; study site included as a random effect.

CHAPTER 4

BROWN-HEADED COWBIRDS EXPLOIT A HOST'S COMPENSATORY BEHAVIORAL RESPONSE TO FECUNDITY REDUCTION

INTRODUCTION

One of the most fascinating outcomes of many host-parasite interactions is the post-infection changes to a host's phenotypes that benefit the parasite's fitness (Thomas et al., 2005; Thomas et al., 2012). Adaptive manipulations of a host's behavior, via physiology or morphology, have been presented as classic exemplars of the 'extended phenotype', where the genes in one organism (i.e. parasite) are selected because of their phenotypic effects on another organism (i.e. host) (Dawkins, 1982). Many parasite-altered phenotypes, however, are likely the result of selection simultaneously acting on both the host's and parasite's genome (i.e. shared phenotype) and therefore, multiple evolutionary routes may have led to adaptive host manipulation (Poulin et al., 1994; Thomas et al., 2012; Wellnitz, 2005). In particular, selection could simultaneously favor both host compensatory efforts that alleviate the fitness costs of infection, and manipulative efforts by parasites that increase parasite fitness (Lefèvre et al., 2009; Lefèvre et al., 2008).

Most organisms are able to cope with changing environments by altering life-history traits to compensate for fitness losses (Metcalf and Monaghan, 2001), including behaviors that alleviate the fitness impact of parasitism (i.e. tolerance) (Svensson and Råberg, 2010). In turn, parasites could achieve adaptive host manipulation by mimicking what induces a compensatory response (e.g. reducing fecundity) in hosts, if the response to infection increases the likelihood of transmission for the parasite (Lefèvre et al., 2009; Lefèvre et al., 2008; Thomas et al., 2012). Because compensation would enable hosts to recoup some parasite-induced fitness loss, the development of host resistance to parasitism would be diminished (Lefèvre et al., 2008; Svensson and Råberg, 2010). Therefore, there is great potential for parasites to increase their fitness by exploiting host compensatory responses, indicating that this type of host-parasite relationship may be widespread.

A few investigations of host manipulation appear to support the ‘exploitation of compensatory responses’ hypothesis. For example, a sexually transmitted ectoparasite, *Chrysomelobia labidomerae*, reduces the survival of infected male host leaf beetles, *Labidomera clivicollis*, and in response, the males increase their sexual behavior (Abbot and Dill, 2001). Therefore, leaf beetles apparently exhibit a compensatory response to infection that provides fitness benefits to parasitized males while also increasing opportunities for parasite transmission (Lefèvre et al., 2008). Recent model simulations indicated that the strategy of exploiting host compensatory responses may be common, particularly in non-trophically transmitted parasite systems (Dubois et al., 2013). However, decoupling host compensatory responses from parasite manipulation strategies and identifying the fitness benefits achieved by both parasite and host is challenging but essential to support the ‘exploitation of host compensatory response’ hypothesis (Thomas et al., 2012).

In response to fecundity reduction, compensatory behaviors likely exist in many songbird hosts of obligate avian brood parasites –species that lay their eggs in a host’s nest and require the parental care of the surrogate parents (Payne, 1977; Rothstein, 1990; Soler, 2014). Tolerant behaviors of hosts, as opposed to resistance, develop in response to the fitness costs of brood parasitism in some cases (Svensson and Råberg, 2010), such as: larger clutch sizes (Soler et al., 2011) or an increase in nesting frequency (Brooker and Brooker, 1996; Hauber, 2003; Smith and Arcese, 1994). In many short-lived bird species, variation in the number of nestlings fledged from a successful nesting attempt may influence the likelihood of initiating another nesting attempt within the same season (i.e. double-brooding) (Parejo and Danchin, 2006). Parental investment beyond the nesting stage (i.e. post-fledging) varies with the number and condition of young and may ultimately influence the decision to double-brood (Linden, 1988). Partial brood reduction (i.e. producing fewer fledglings), a result of either experimental manipulations (Linden, 1988; Parejo and Danchin, 2006) or natural causes (Nagy and Holmes, 2005), may increase the likelihood of double-brooding while pairs with enlarged brood sizes are less likely to initiate an additional breeding attempt. By affecting the total number of fledglings produced, brood parasites could affect the frequency of double-brooding in host species capable of double-brooding. Brood parasites reduce the host’s

fecundity by adding one or more parasitic eggs to the host's clutch, removing host eggs (Davies and Brooke, 1988; Hoover, 2003c; Scott et al., 1992), reducing hatching success (Smith and Arcese, 1994; Soler et al., 1997), and reducing nestling survival of hosts when simultaneously raised with parasites (Hoover, 2003c; Soler and Soler 1991; Vernon 1964). Host nestlings with less competitive ability than their parasitic nestmates often perish; and this competition for parental care likely continues during the fledgling stage, resulting in increased mortality of host fledglings (McKim-Louder et al., 2013; Peterson et al., 2012; Rasmussen and Sealy, 2006). Hosts producing a reduced total number of fledglings from their first nesting attempt, regardless of parasitism status, may be more likely to double-brood (i.e. initiate an additional clutch) in an attempt to counterbalance any fecundity reduction during the first nesting interval (Linden, 1988). An increase in host double-brooding (i.e. compensation) may provide brood parasitic females, which typically use consistent egg laying ranges during the breeding season (reviewed in Hauber and Dearborn, 2003), additional reproductive opportunities (i.e. exploitation).

Brood parasitism, alternatively, could reduce the likelihood of hosts making additional nesting attempts. By definition brood parasitism is energetically costly to the host as parasitic nestlings generally demand increased amounts of resources relative to the host offspring (Hoover and Reetz, 2006; Mark and Rubenstein, 2013; but see Canestrari et al., 2014). Parasitic nestlings typically weigh much more than host nestlings, often more than the combined weight of the entire host brood. In turn, brood parasites may actually decrease the chance of double-brooding as the costs of rearing an energetically demanding parasite diminishes the host parents' ability to reproduce again.

Avian brood parasite-host interactions provide a unique opportunity to test the 'exploitation of host compensatory response' hypothesis by comparing similar stressors (i.e. fecundity reduction) between non-parasitized and parasitized nests, and therefore, separating the parasite's transmission strategy from the host's response. Here we investigate the impact of brood parasitism by brown-headed cowbirds (*Molothrus ater*) on the probability of double-brooding in an individually-marked population of a multiply-brooding host species, the prothonotary warbler (*Protonotaria citrea*). Using data collected from both parasitized and non-parasitized successful nests, we examine the effects of fecundity reduction and

brood parasitism on the probability adult female warblers initiate an additional clutch within the same season. We predict that: (i) natural levels of fecundity reduction (e.g. hatching failure, nestling death) would increase the likelihood of double-brooding in non-parasitized nests, and (ii) fecundity reduction attributable to brood parasitism, measured during the nesting stage, would stimulate a similar compensatory response, increasing the probability of double-brooding in the host. Specifically, we predicted that female warblers with parasitized nests would fledge the least offspring yet have a greater likelihood to double-brood. Similarly, we predicted that female warblers associated with experimentally parasitized nest boxes would double-brood at a greater frequency when compared to females using nest boxes where all effects of cowbirds were excluded. We also used radio-telemetry to determine the fate of 26 cowbird offspring to account for potential effects of post-fledging cowbird mortality on double-brooding in the host. Both female cowbirds (Hahn et al., 1999) and warblers (Hoover, 2003a) breed within their respective territories throughout the year, thus additional warbler breeding attempts would provide additional parasitism opportunities. We compared parasitism rates during the additional breeding attempts, using the parasitism status in the first brood as a predictor to identify non-random parasitism in the subsequent brood indicative of realized enhancement of transmission.

METHODS

Study population and field methods

During 1994-2012, we studied a population of prothonotary warblers located in the Cache River Watershed, Illinois, USA (37°18'N, 88°58'W) that is commonly parasitized by the brown-headed cowbird (Hoover, 2003b, c; Hoover and Reetz, 2006). The prothonotary warbler is a Neotropical migratory songbird that inhabits flooded bottomland forests and swamps throughout eastern North America (Petit, 1999). This species is territorial, socially monogamous (Petit, 1999) and as a secondary cavity nester, readily uses nest boxes when provided. Successful breeding pairs are highly territory faithful within a season, initiating additional clutches in the same or neighboring nest boxes (Hoover, 2003a). Typically double-brooded, prothonotary warblers lay 4-5 eggs during each nesting attempt (Petit, 1999). As a host, the prothonotary warbler appears to lack adaptive responses to brood parasitism (Hoover, 2003b) and

while able to raise a few warbler offspring with a cowbird nestling, parasitism events typically result in a reduction of host fecundity (Hoover, 2003c).

Common throughout North America, brown-headed cowbirds are obligate brood parasites that have been successfully raised by ~144 host species (Lowther, 1993). Telemetry (Dufty, 1982; Raim, 2000) and genetic studies (Hahn et al., 1999; Rivers et al., 2012) have demonstrated that female cowbirds have a specific egg-laying range throughout a season. On average, female cowbirds remove a single prothonotary warbler egg from about 60% of nests that are parasitized in our study system and cowbird nestlings increase host offspring mortality, ultimately reducing the number of warblers fledging (Hoover, 2003c). In prothonotary warbler nests, cowbird nestlings increase the rate of food provisioning by adult warblers (Hoover and Reetz, 2006) and, weigh on average 2-3 times more than warbler nestlings (Hoover, 2003c).

Each year, approximately 1500 nest boxes (1.9 L cardboard milk carton; 95 mm x 95 mm x 200 mm) were placed 40-50 m apart within appropriate habitat in approximately 20 study sites. Nest boxes were placed 1.7 m above ground and had 44-mm-diameter openings, comparable to the attributes of natural cavities used by warblers in this study system (Hoover, 2001). To limit nest predation, a majority of the nest boxes were attached to greased conduit poles. We monitored nest boxes every 3-6 days throughout the breeding season and we recorded the number of eggs and nestlings of warblers and cowbirds present at each visit. Nest initiation dates (i.e. first day of incubation) were calculated by estimating nestling ages and backdating 12 days for incubation (Petit, 1999). We assumed nestlings fledged if they reached 10-11 days of age and the nest was empty and intact. Additional evidence of fledging included the presence of trampled droppings in the nest, alarm calls from adults, and observations of appropriately aged fledglings in the territory. Adult warblers were captured and fitted with a unique color-band combination and a numbered aluminum band. The ages for female warblers were identified by wing and tail feather criteria (Pyle, 1997); aged as either second-year (i.e. 1-year old; labeled as 1) or after-second-year (i.e. > 1 year old; labeled as 2). The identities of ca. 90% of the adults from each nesting attempt were recorded by either capture or visual identification.

Experimental parasitism

In 2006, we devised an experiment where warbler nests were randomly chosen to be parasitized or not on six study sites to eliminate the potential confound that female cowbirds selectively parasitize female warblers that have a greater likelihood of double-brooding. During May, we experimentally added two non-incubated cowbird eggs to approximately half of the active nest boxes while female warblers were still laying. We added two eggs to account for cowbird hatching failure and to ensure experimentally parasitized nests fledged at least one cowbird. Opening sizes of nest boxes were made to be small enough (38 mm) to exclude most female cowbirds, but still large enough to encourage use by the warblers. If female cowbirds, in general, selectively parasitize female warblers that are more likely to double-brood, then our random assignment of parasitism status should have eliminated this confound and resulted in two treatment groups that were not different in the probability of double-brooding.

Radio-telemetry

To determine whether cowbird post-fledging survival influences the double-brooding decisions of warbler hosts, we attached radio-transmitters (0.9 g) to nestling cowbirds just prior to fledging (post-hatch day 10) from warbler nest boxes on two study sites during May, 2013. We located cowbird fledglings daily throughout the life of each transmitter (battery life ~ 20 days; Sparrow Systems) with a handheld 3-element Yagi antennae and receiver. Evidence indicating juvenile cowbird mortality included plucked feathers and bite marks on recovered transmitters. Post-fledging mortality generally occurs within the first 3-weeks after fledging, typically during the first few days (Cox et al., 2014), therefore we assumed cowbirds reaching 20 days old survived to reach independence from the host.

Statistical Analyses

All statistical analyses were performed in SAS (SAS 9.4, Cary, NC, USA) and parameter estimates are presented with \pm standard errors. To identify the factors correlated with double-brooding within the non-experimental dataset, we used generalized linear mixed models (GLMM) with a binomial response, logit link function, and Laplace likelihood approximation (Bolker et al., 2009). For these analyses, we limited the potential effects of making multiple nesting attempts on females and their ability

to double-brood by only including successful nests initiated from the beginning of the breeding season to June 1. We estimate that before June 1, most (> 85%) of these nests were females' first nesting attempts for a given year. There could be some females in this sample that are re-nesting after early nest failure (i.e. egg stage) but we did not want to exclude first attempts by younger females that typically arrive later on breeding grounds. The potential effect of non-independence for the same female nesting across several years was controlled for by including female warbler identity as a random variable. Furthermore, GLMMs investigating the factors correlated with double-brooding probability included the effects of year (categorical), nest initiation date (ordinal) and female warbler age (continuous) (Bulluck et al., 2013; Townsend et al., 2013) as covariates. Correlation coefficients of explanatory variables were investigated prior to analysis to ensure that correlated explanatory variables ($r > 0.7$) were not used in the same analysis.

Fecundity reduction and double-brooding

To determine if the likelihood of double-brooding for female warblers is correlated with fecundity reduction, we used a model including only non-parasitized nests and examined the relationship between residual brood size (number of fledglings – number of warbler eggs) and the probability of initiating a second clutch. Furthermore, we included the number of warbler eggs into the model as a potential explanatory variable to ensure that the number fledging from the first brood reflects fecundity reduction rather than variation in female's initial clutch size.

Brood parasitism and double-brooding

Because warblers fail to recognize foreign eggs (Hoover, 2003b) and provide care for cowbird offspring throughout the nestling and post-fledging stages, even at the expense of adult and juvenile warbler survival (Hoover and Reetz, 2006; McKim-Louder et al., 2013), we assume a warbler's 'perceived fecundity' includes the number of both warbler and cowbird fledglings. Therefore, we estimated the effect of parasitism on perceived fecundity with a GLMM that included the total number of fledglings (both cowbird and warbler) as the dependent variable (normal distribution) and the parasitism

status as an independent variable, while including female warbler identity as a random effect and controlling for the fixed effects of year (categorical), date, and female age.

We then used a GLMM to compare the probability of double-brooding among successful parasitized nests that fledged at least one cowbird offspring and non-parasitized nests that fledged at least one warbler offspring, while controlling for the potential effects of female identity (random effect) and including date, year, and warbler age as covariates. For the experimental data collected in 2006, we used a Pearson chi-square test to compare the frequency of double-brooding among experimentally parasitized broods that fledged at least one cowbird with nest boxes where no cowbird eggs were added (i.e. non-parasitized).

To identify if double-brooding females that raised a cowbird in their first brood are more likely to be parasitized again, thus enhancing parasite transmission, we compared the parasitism rates of the second breeding attempt between double-brooded females that were either parasitized or non-parasitized during their first brood. We examined the parasitism status during the second breeding attempt (binomial response variable) using a GLMM with female identity included as a random effect while including year and initiation date of the second brood as covariates.

RESULTS

Fecundity reduction and double-brooding

To investigate the effects of fecundity reduction on double-brooding, we used a GLMM that included non-parasitized first broods where the number of warbler eggs were known ($n = 761$), while controlling for female identity ($n = 627$) as a random effect and year (range = 1994-2012, $F_{18,125} = 2.68$, $P < 0.001$), nest initiation date ($F_{1,125} = 47.96$, $P < 0.001$), warbler age ($F_{1,125} = 12.75$, $P < 0.001$) as covariates. In support of hypothesis (i), the probability of double-brooding in non-parasitized nests increased with lower residual brood size (number of fledglings – number of warbler eggs) (GLMM: $\beta = -0.44 \pm 0.11$, $F_{1,125} = 15.08$, $P < 0.001$). On average, non-parasitized female warblers fledging four less offspring were twice as likely to attempt an additional brood when compared to non-parasitized females that fledged all offspring (Fig. 4.1). Therefore, in the absence of brood parasitism female prothonotary

warblers appear to compensate for fecundity reduction in their first successful brood by attempting a subsequent clutch. The clutch size had less influence on the probability of double-brooding (GLMM: $\beta = -0.26 \pm 0.15$, $F_{1,125} = 3.13$, $P = 0.08$). The number of warbler fledglings was significantly correlated with residual brood size in non-parasitized nests (Pearson correlation: $r = 0.76$, $P < 0.001$), indicating that the number of fledglings reflects a suitable measure of fecundity reduction.

Parasitism and double-brooding

The combined parasitized and non-parasitized dataset included 942 individual female prothonotary warblers associated with 1245 successful first broods where incubation was initiated prior to June 1 within a given year, of which 589 (46%) were followed by an additional clutch (i.e. double-brooding). The parasitism status of a female warbler was found to significantly influence the number of offspring produced (GLMM: $F_{1,282} = 66.21$, $P < 0.001$), where the predicted mean actual fecundity in non-parasitized nests (warbler offspring = 4.22 ± 0.05) was greater than the predicted mean ‘perceived fecundity’ (warbler and cowbird offspring = 3.61 ± 0.06) in nests that fledged at least one cowbird. Similarly, perceived fecundity was reduced in experimentally parasitized nests (mean = 3.91 ± 0.26) compared to actual fecundity in non-parasitized nests (mean = 4.25 ± 0.09) ($t = 6.84$, $df = 1$, $P < 0.001$), thus experimentally demonstrating a negative effect of brood parasitism on perceived fecundity.

A female warbler’s brood parasitism status (GLMM: $F_{1,282} = 6.82$, $P = 0.01$) significantly explained variation in her probability of double-brooding within the non-experimental dataset ($n=1245$), while controlling for the confounding variables of year ($F_{18,282} = 5.90$, $P < 0.001$), nest initiation date ($F_{1,282} = 319.31$, $P < 0.001$), and female warbler age ($\beta = 0.19 \pm 0.05$, $F_{1,282} = 20.75$, $P < 0.001$). Females that fledged at least one cowbird were significantly ($\beta = 0.38 \pm 0.15$, $t_{1,282} = 2.61$, $P = 0.01$; Fig. 4.2) more likely to double-brood (0.55 ± 0.03) than non-parasitized females (0.46 ± 0.02).

With small nest box opening sizes (38 mm), we experimentally excluded cowbird females from 107 nest boxes that successfully fledged offspring, of which 43 were randomly selected to receive two cowbird eggs during the egg laying stage. Eight experimentally parasitized nests failed to fledge any

cowbirds because of hatching failure and were removed from the analysis. Experimentally parasitized females that fledged at least one cowbird ($n=35$) were significantly more likely to double-brood than non-parasitized female warblers ($n = 64$) ($\chi^2 = 4.25$, $df = 1$, $P = 0.04$; Fig. 4.3). Of note, double-brooding frequencies for experimental data reflect nests during 2006 only, whereas results from the correlative dataset (Fig. 4.2) includes the mean predicted double-brooding probabilities averaged across all years.

In 2013, radio-transmitters were attached to 26 juvenile cowbirds prior to fledging, of which 8 (31%) survived to 20 days post-fledging and were presumed to reach independence from the warbler host. All radio-tagged juvenile cowbirds fledged from nests initiated during May. The frequency of double-brooding was identical (50%) among female warblers of nests where cowbirds died quickly (i.e. within 1-2 days) after fledging and nests that successfully raised cowbirds to independence from the host (Fisher's exact: $\chi^2 = 0.00$, $df = 1$, $P=1$).

Probability of parasitism between consecutive clutches

Nearly all female warblers (90%) that initiated nests prior to June 1 and double-brooded, used the same or adjacent nest box (< 100m away) between consecutive clutches. To determine whether the increased frequency of double-brooding by parasitized female warblers translated into transmission benefits for female brown-headed cowbirds, we used the parasitism status in the first warbler nest to predict the probability of parasitism in the second nest. Parasitism status in the first nest positively influenced the probability of parasitism in the second nest (GLMM: $n = 328$, $F_{1,19} = 26.96$, $P < 0.001$), controlling for the effects of female (random effect), year ($F_{18,19} = 1.28$, $P = 0.19$) and the initiation date of the second brood ($\beta = -0.02 \pm 0.03$, $F_{1,19} = 0.39$, $P = 0.53$). Warblers parasitized in the first brood were more than seven times as likely to be parasitized again in the second breeding attempt (0.47 ± 0.26) as those not parasitized in their first attempt (0.06 ± 0.06); this non-random parasitism provides support for our hypothesis that parasites gain transmission benefits from these second nesting attempts.

DISCUSSION

In this study, we demonstrate an indirect post-infection alteration of host behavior in an avian host-parasite relationship where the likelihood of attempting a second brood in female prothonotary

warblers increases in response to brood parasitism by brown-headed cowbirds. A significant increase in the double-brooding frequency for parasitized female warblers was identified within a long term observational dataset (19 years), while accounting for the potential confounding effects of annual variation in double-brooding frequency, female warbler age and date within the season. Experimental evidence provided additional support for the correlative result, indicating that the increase in double-brooding is an effect of brood parasitism and not cowbirds selectively parasitizing female warblers that are more likely to double-brood. In non-parasitized nests that successfully fledged at least one warbler offspring, the likelihood of double-brooding increased with fecundity reduction caused by hatching failure and nestling death. By reducing the host's fecundity, cowbirds induced a similar double-brooding response and thus elicited a compensatory behavior that enabled warblers to recover a portion of fecundity lost during the first brood while providing additional opportunities for parasitism. Furthermore, evidence from our study system (Hoover and Reetz, 2006) and others (Payne and Payne, 1998; Smith, 1981), indicate that the impact of raising 1-2 cowbirds on adult host survival is negligible, and therefore, that the benefits of compensatory double-brooding in response to parasitism are not diminished by reduced adult host survival.

Relatively high cowbird post-fledging mortality, rather than reduced fecundity, could increase compensatory double-brooding of hosts if the loss of the cowbird fledgling is viewed by the host as a further reduction in fecundity. This could lead to spurious conclusions regarding the factors guiding a host's behavior in response to parasitism. However, results from radio-telemetry indicated that female warblers double-brooded at the same frequency regardless of fledgling cowbird mortality. Alternatively, the decision to double-brood could be influenced by a shorter post-fledging period if warbler parents spend less time caring for parasite offspring. If cowbirds reach independence earlier than host offspring, then double-brooding rates could increase for hosts with parasitized first broods. However, the duration of post-fledging care for prothonotary warbler parents appears to be similar when caring for either warbler offspring (< 35 days, (Petit, 1999)) or a cowbird (< 33 days; M. Louder, unpublished data). Therefore,

these alternative hypotheses are unlikely explanations for the observed double-brooding response of female warblers rearing parasitized broods.

Because adult females of both warblers (Hoover, 2003a) and cowbirds (Hahn et al., 1999) are territory faithful throughout the breeding season, warblers are likely parasitized in sequential broods by the same female cowbird. Some double-brooding female warblers escape parasitism during second broods because of reduced activity by the parasite, resulting in a potential fitness benefit of double-brooding in response to parasitism. Yet, on average 48% of first broods that fledge cowbirds will be parasitized again in the second brood. Hosts attempting second broods to compensate for fecundity reduction attributable to brood parasitism provide additional transmission opportunities that are exploited by adult female cowbirds while yielding some additional warbler offspring from non-parasitized and even parasitized nests. Adaptive behavioral modifications within interspecific avian brood parasite-host systems are well-documented phenomena (Davies, 2000), but to our knowledge, this is the first study to suggest that brood parasites could gain transmission benefits by reducing host fecundity.

Compensatory double-brooding behavior in response to natural levels of fecundity reduction may indeed be widespread in songbirds, resulting in extensive opportunities for exploitation by brood parasites. Brown-headed cowbirds have successfully coerced at least 144 species into raising parasitic offspring (Lowther, 1993), many of which double-brood. A comparison of species-specific double-brooding rates by Hauber (2003) revealed that evolutionarily 'old' host species, those with longer historical host-cowbird associations, tend to double-brood more than novel host species. This relationship may be a result of selective pressures favoring hosts that alleviate the costs of parasitism by double-brooding, while simultaneously favoring brood parasites that lay eggs in additional host nesting attempts. Without directly controlling for the effects of date or brood size, double-brooding probability was found to be 19% greater in parasitized than non-parasitized indigo bunting (*Passerina cyanea*) nests at one study site (Payne and Payne, 1998). Similarly, an increase in double-brooding was suggested in a host of the Horsfield's bronze-cuckoo (*Chrysococcyx basalis*) (Brooker and Brooker, 1996), yet, bronze-cuckoos evict all host eggs/nestlings and brood size and date would also need to be accounted for in determining

the host's reneating decisions. Future studies monitoring the reproductive success of both host and brood parasitic individuals will help elucidate the role of host compensatory behaviors in avian brood parasite transmission strategies and provide insight into the evolution of host manipulation.

Although host manipulation is generally regarded as an extended phenotype of the parasite, multiple evolutionary routes have potentially led to manipulated host behavior. Many parasite-altered phenotypes may actually reflect the parasite *and* host's strategies selected to achieve fitness benefits rather than merely parasite enforced behaviors. Among the various abilities to compensate for fitness loss, including tolerant behaviors in response to parasitism (Svensson and Råberg, 2010), it is likely that at least some tactics hosts employ to alleviate infection will also benefit the parasite. Selection should simultaneously favor both host behaviors that compensate for fecundity reduction and the parasite's exploitation of host compensatory behaviors if aligned with transmission pathways (Lefèvre et al., 2009; Lefèvre et al., 2008; Thomas et al., 2012). Recent simulation models have revealed that exploitation of compensatory responses should be a widespread strategy, particularly in non-tropically transmitted parasites (Dubois et al., 2013). By exploiting a host's response to fecundity reduction, the parasite's manipulative efforts are minimized; the parasite merely has to reduce fecundity, something it already does. Furthermore, selective pressures on the development of host resistance to parasitism are reduced as hosts achieve some fitness benefits after compensating for the effects of parasitism (Canestrari et al., 2014; Lefèvre et al., 2008; Svensson and Råberg, 2010). Additional analyses of host-parasite relationships are likely to reveal more situations where both parasites and hosts are able to achieve some fitness benefits.

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FIGURES

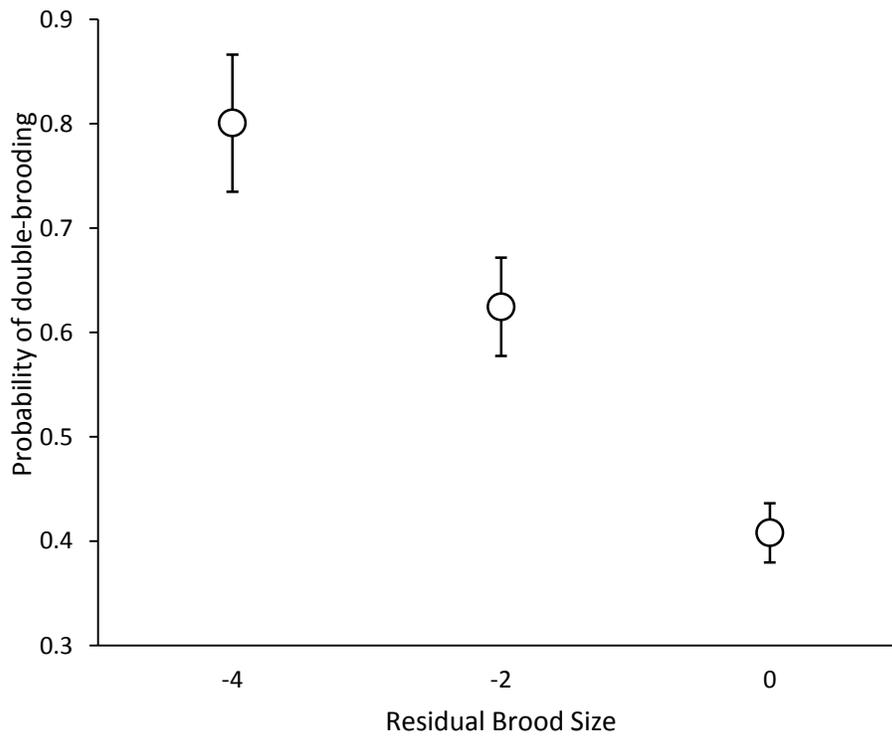


Figure 4.1.
Relationship between the probability of non-parasitized female warblers to double-brood ($n=761$) and fecundity reduction, measured as the residual brood size (number of fledglings – number of warbler eggs). Predicted probabilities (\pm s.e.) of double-brooding shown from results of a GLMM while holding covariates at mean observed values.

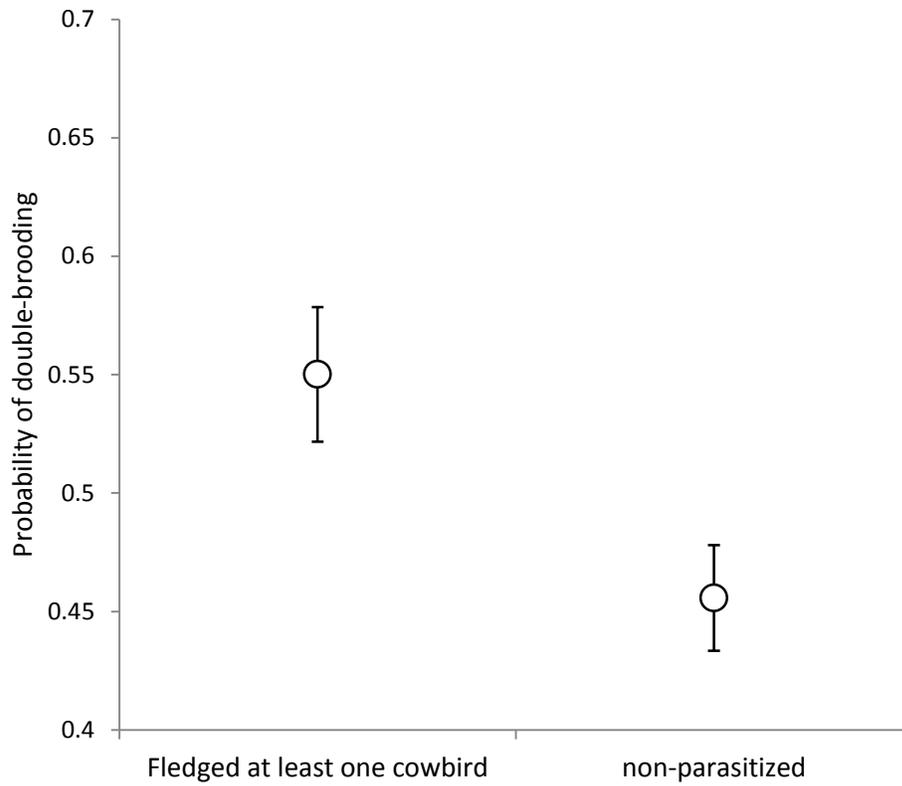


Figure 4.2. A comparison of the probability of double brooding between female warblers parasitized (fledged at least one brown-headed cowbird offspring) and non-parasitized during their first brood. Predicted probabilities presented (\pm s.e.) from a GLMM while holding covariates at mean observed values.

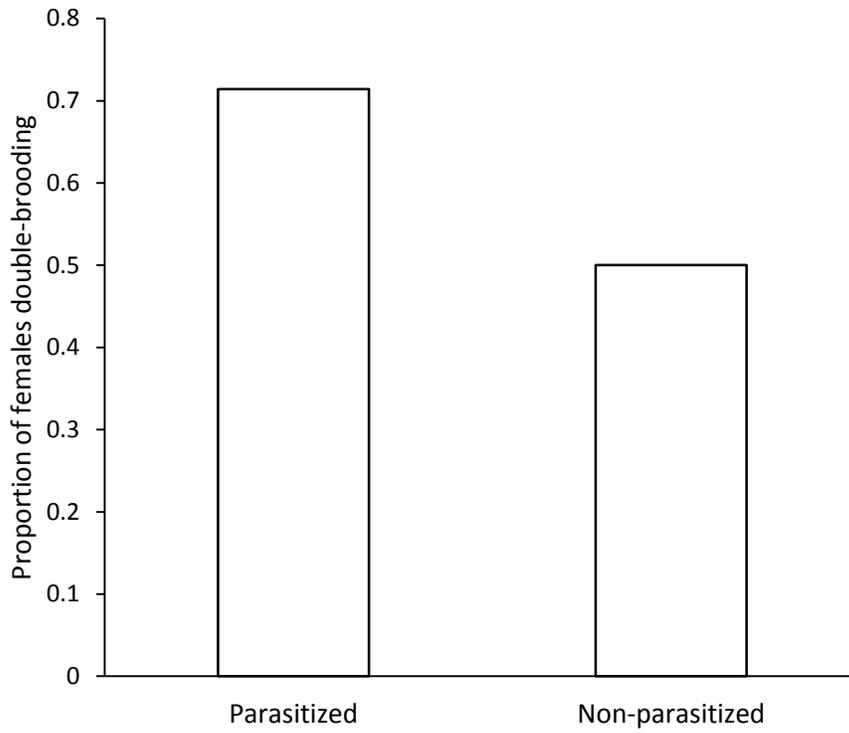


Figure 4.3.
The proportion of female prothonotary warblers double-brooding for experimentally parasitized (n= 35) and non-parasitized (n = 64) first broods during May 2006.

CHAPTER 5

SUMMARY

Parasites have evolved from non-parasite ancestors hundreds of times in the eukaryotes, yet our understanding of the evolution and persistence of parasites-host interactions remains incomplete (Poulin 2011). Although parasites are extant in nearly every taxonomic group, each parasite must overcome similar selective pressures, such as survival when in association with the host, choosing an appropriate host and sustainably exploiting the host's resources (Poulin and Randhawa 2015). Avian brood parasites provide opportunities to directly observe behaviors and fitness relationships among parasites and their hosts, which are often difficult to quantify within host-parasite systems in nature (Rothstein 2010; Feeney et al 2014). With that in mind, the goal for my dissertation was to identify strategies that obligate parasitic brown-headed cowbirds (*Molothrus ater*) employ to complete their reproductive cycle (i.e. develop from offspring to adulthood), choose high quality hosts, and minimize virulence when exploiting the host's parental care.

Sexually imprinting on the foster species is considered one of the greatest constraints to the evolution of interspecific brood parasitism (Slagsvold and Hansen 2001). Where non-parasitic juvenile birds learn the behaviors and mate choice preferences of their parents (reviewed in ten Cate and Vos 1990), parasites must avoid mis-imprinting on their host's phenotype to recognize conspecifics (Slagsvold and Hansen 2001; Sorenson et al. 2010). The 'first contact hypothesis' proposes that juvenile cowbirds may innately prefer their own species and follow adult female cowbirds from the host to social foraging flocks (Hauber 2002). Yet, I demonstrate in chapter 2 that juvenile cowbirds typically depart the forest at sunset and roost solitarily. This result suggests that cowbirds may use an innate roosting preference to minimize imprinting on the host; pointing to the importance of inherited preferences within the evolution of brood parasitism. Future research should focus on the significance of innate habitat preferences in locating conspecifics and the neural processes that enable brood parasites to recognize their own kind.

As adults, brood parasites should prefer hosts that have the greatest potential to raise parasitic offspring (Soler 2014). Although a host's likelihood of receiving a parasitic egg is often correlated with host quality (reviewed in Parejo & Avilés 2007; Feeney et al. 2012), the role of learning in parasite's reproductive decisions had previously not been investigated. By studying the behaviors of juvenile cowbirds and their effects on host-parasite interactions within a single host species, the prothonotary warbler (*Protonotaria citrea*), I eliminated inter-specific variation in the ability to raise cowbird offspring that would otherwise obscure the ability to detect the parasite's decisions. In chapter 3, I demonstrated that cowbird fledglings serve as a cue used by adult female cowbirds to prefer high quality hosts and avoid relatively poor hosts. Non-parasitic bird species often use reproductive performance information to influence reproductive decisions, increasing spatial fidelity in nest-site choice in response to reproductive success of their own and neighboring individuals (Danchin et al. 2004; Schmidt et al. 2010). The preference for high quality hosts, while simultaneously avoiding poor hosts, may influence the coevolutionary dynamics of host-parasite relationships. A preference for a host could increase the selective pressure defense strategies to develop. On the other hand, reduced parasitism pressure for poor hosts could effectively delay the development of host resistance when initiated within a given population, as the rate of development and spread of parasite rejection behaviors for hosts depends on the parasitism frequency (Takasu 1998). Future host-parasite models that introduce learning have the potential to enhance our understanding of the evolution of host specificity and the effect of parasite cognition on the development of anti-parasite defenses in hosts.

By minimizing virulence, parasites may further decrease the selective pressures for the development of host resistance (Svensson and Råberg 2010; Medina and Langmore 2015). A recent hypothesis proposes that parasites may induce compensatory responses in hosts that, in turn enable hosts to recoup some fitness lost to parasitism while facilitating parasites to achieve successful transmission (Lefèvre et al. 2008). My results from chapter 4 indicate that raising juvenile cowbirds at a fecundity loss for female warblers increases their likelihood of initiating a second brood (i.e. double-brooding). Experimentally parasitized female warblers exhibited a similar increase in double-brooding behavior, and

the double-brooding frequency of parasitized female warblers was not attributable to reduced post-fledgling survival of cowbirds; thereby supplying further support for the role of parasite-induced fecundity reduction in the compensatory double-brooding behavior of a host. Evolutionarily ‘old’ host species, those with longer historical host-cowbird associations, tend to double-brood more than novel host species (Hauber 2003). Therefore, selective pressures may favor hosts that alleviate the costs of parasitism by double-brooding, while simultaneously favoring brood parasites that lay eggs in additional host nesting attempts. Future evolutionary modelling, that incorporates the fitness benefits and costs of this relationship, will help reveal whether compensatory double-brooding induced by fecundity reduction adaptively minimizes the development of anti-parasites defenses.

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