LIFE HISTORY, STRESS HORMONES, AND EGG VISUAL SIGNALS AS PREDICTORS OF CLUTCH ABANDONMENT IN HOSTS OF BROOD-PARASITIC COWBIRDS

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Abstract

During reproduction, parents invest time and resources in their offspring. Situations in which a clutch requires a parent to invest too many resources can lead to clutch abandonment. This response can be adaptive or not depending on the relative importance of the clutch compared to the lifetime reproductive output of the parent. We define the relative importance of a clutch as its brood value. In the context of obligate brood parasitism, where a parasite lays their eggs in the nests of other species, some hosts use clutch abandonment as an antiparasitic response. In this thesis, I explore two perspectives that can explain variation in this response. First, I take a life history point of view. Brood value, as well as factors such as habitat and host sensitivity, are hypothesized to explain differences in clutch abandonment rates in parasitized nests, as they change the cost of abandonment in different species. I compiled clutch abandonment rates from the literature in hosts of the *Molothrus* genus of parasites, and coupled them with brood value and life history traits. I found that clutch abandonment is predicted by brood value only in species with reduced exposure to brood parasitism, a relationship which only held in the absence of parasitism. This suggests that brood parasitism overrides what factors predict clutch abandonment. My second perspective takes a more mechanistic approach, looking at the hormonal and visual processes involved in mediating the decision to abandon in one common host species, the yellow warbler (*Setophaga petechia*). I found that corticosterone (CORT), an important stress response hormone in birds, was in higher levels in individuals that abandoned than those that did not abandon their nest, whereas CORT was similar between unparasitized birds and those that accepted parasitism. Meanwhile, feather-extracted long-term deposited CORT and egg maculation characteristics were not associated with abandonment. Tackling these questions will contribute to the understanding of clutch abandonment, and more broadly to how parental care is modulated.

Résumé

Lors de la reproduction, les animaux investissent beaucoup de temps et de ressources dans leur progéniture. Les situations qui font que le coût d'une couvée nécessite un investissement trop grand du parent peuvent conduire à l'abandon de la couvée. Cette réaction peut être adaptative ou non en fonction de l'importance relative de la couvée par rapport à l'effort reproductif total pendant la vie du parent. Cette importance relative défini le *brood value*, la valeur de la couvée. Dans le contexte du parasitisme de couvée, où un parasite pond ses œufs dans les nids d'une autre espèce, certains hôtes utilisent l'abandon de couvée comme réponse antiparasitaire. Dans la présente thèse, j'explore deux perspectives qui peuvent expliquer cette réponse. Premièrement, je considère la perspective de l'histoire de la vie. La valeur de la couvée, ainsi que des facteurs tels que l'habitat et la sensibilité de l'hôte pourraient expliquer les différences dans les taux d'abandon de couvée dans les nids parasités, car ils modifient le coût de l'abandon chez différentes espèces. J'ai examiné les taux d'abandon des couvées en raison du parasitisme notés dans la littérature chez les hôtes de parasites du genre *Molothrus*, et je les ai couplés avec la valeur des couvées calculée à partir du succès de reproduction à vie. J'ai constaté que l'abandon de la couvée est prédit par la valeur de couvée uniquement chez les espèces avec une exposition réduite au parasitisme du couvain, une relation qui ne tenait qu'en l'absence de parasitisme, ce qui suggère que le parasitisme de couvée change de manière significative voire remplace les facteurs prédisant l'abandon de la couvée. La seconde perspective, quant à elle, adopte une approche plus mécaniste en examinant les processus hormonaux et visuels impliqués dans la médiation de la décision d'abandonner chez une espèce hôte particulière, la paruline jaune (*Setophaga petechia*). J'ai constaté que la corticostérone (CORT), une hormone du stress chez les oiseaux, se trouvait à des niveaux plus élevés chez les oiseaux qui abandonnaient, tandis que chez ceux qui acceptaient le parasitisme elle était comparable à celle des oiseaux non parasités. Cependant, le CORT extrait des plumes, qui mesure les niveaux de CORT à long terme, de même que les différences de maculation entre les œufs de parasite et ceux des parulines, ne sont pas associés avec les abandons. S'attaquer à ces questions contribuera à une meilleure compréhension des comportements parentaux en matière de soins ainsi que de la façon dont l'investissement parental est modulé.

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Contribution to original knowledge

This thesis is manuscript based, with all Chapters containing original scholarship and contributions to knowledge. Chapter 2 and 3 address topics of current interest, in new and promising ways. In Chapter 2, to the best of my knowledge, I provide evidence for the first time that clutch abandonment and brood value are inversely related in the absence of parasitism, but that brood parasitism overrides the effect of brood value on clutch abandonment. I also find that the rate of clutch abandonment in response to parasitism differs depending on the degree of parasite-host interaction as measured by habitat differences, host sensitivity and migratory status. In Chapter 3, I manipulated clutches by adding model parasitic eggs in the nests of yellow warblers (*Setophaga petechia*), a host species known to regularly abandon clutches parasitized by brown-headed cowbirds (*Molothrus ater*) via egg burial and nest desertion. I show that baseline corticosterone levels were elevated in individuals who abandoned their experimentally parasitized clutch, but not in individuals who accepted, compared to controls. To the best of my knowledge, I offer the first evidence of a link between elevated baseline plasma corticosterone and clutch abandonment in a host species relying only on clutch abandonment to respond to parasitism at the egg-laying stage. Overall, these results provide a broad overview of some of the predictors of clutch abandonment and documents ways that parental care investment is impacted by brood parasitism.

Contribution of authors

Chapter 1: General Introduction

Antoine V. Turcotte-van de Rydt wrote the chapter with guidance and input from Mélanie F. Guigueno.

Chapter 2: A meta-analysis of life history traits affecting clutch abandonment in cowbird hosts: parasitism overrides brood value in environments with higher parasite-host interactions

Antoine V. Turcotte-van de Rydt and Mélanie F. Guigueno designed the study. Antoine V. Turcotte-van de Rydt and Christina Petalas collected data. Simon Ducatez and Antoine V. Turcotte-van de Rydt analyzed data. Antoine V. Turcotte-van de Rydt and Christina Petalas wrote the manuscript with input from Simon Ducatez and Mélanie F. Guigueno. Manuscript to be submitted to the Proceedings of the Royal Society B: Biological Sciences. All authors approve submission as part of the present thesis.

Chapter 3: Clutch abandoning parasitized yellow warblers have increased baseline corticosterone with no effect of past corticosterone or differences in egg maculation characteristics

Antoine V. Turcotte-van de Rydt, Mélanie F. Guigueno and Sharon A. Gill designed the study. Antoine V. Turcotte-van de Rydt, Christina Petalas, Joanna M. Sblendorio, Sharon A. Gill and Mélanie F. Guigueno conducted the experiment and collected data. Antoine V. Turcotte-van de Rydt, Christina Petalas, Christopher A. Pearl and Mélanie F. Guigueno analyzed data. Antoine V. Turcotte-van de Rydt and Christina Petalas wrote the manuscript with guidance from Mélanie F. Guigueno and Sharon A. Gill. Manuscript under minor revision at Frontiers in Ecology and Evolution. Authors approve submission as part of the present thesis.

Chapter 4: General discussion

Antoine V. Turcotte-van de Rydt wrote the chapter with guidance from Mélanie F. Guigueno.

CHAPTER 1: GENERAL INTRODUCTION

The cost and benefits of reproduction and parental care is one of the most widely studied fitness trade-offs and is fundamental in understanding life-history evolution (Linden and Møller 1989; Nilsson and Svenssonn 1996; Harshman and Zera 2007; Rauset et al. 2015; Richardson et al. 2020). Reproduction is a central aspect of the life cycle of animals, and its success can be increased by parents investing resources and time into the rearing of offspring (Williams 1966; Trivers 1972; Gross 2005; Davies et al. 2012). Parental investment refers to the costs parents expend to increase the fitness of their offspring, which includes everything from the production of gametes, nest building, and care and protection of their offspring (Trivers 1972), whereas parental care refers to the subset of parental investment adaptations improving offspring fitness after fertilization (Gross and Shine 1981). Parental investment is costly to the parents and can negatively impact subsequent survivability and fertility (Mark and Rubenstein 2013; Williams 2018). Studies that experimentally manipulate brood sizes show that increasing the number of offspring often results in future fitness costs for the parents like reduced body condition and lifespan (Velando and Alonso-Alvarez 2003; Santos and Nakagawa 2012). Parental care decisions therefore involve trade-offs between allocation of resources to current reproduction effort and to future reproductive efforts (Remick 1992), as the offspring seek to maximize care for themselves and the parents seek to invest equally in all broods (Figure 1.1; Trivers 1974; Lazarus and Inglis 1986). As Figure 1.1 illustrates, the optimal parental investment that maximizes the benefit to offspring occurs at a larger cost than the investment that maximizes the benefit to the parent. Consequently, there is necessarily a conflict between the two and the investment of the parent in the offspring will always be a compromise.

Brood value

Parental care varies based on a parent's perceived value of a brood (Williams 1966; Gross 2005). The importance of a brood can be quantified as brood value, defined as (Bókony et al. 2009; Sol et al. 2016):

$$\log\left(\frac{clutch\ size}{clutch\ size\ \times broods\ per\ year\ \times maximum\ reproductive\ lifespan}\right)$$

This equation represents an integrated measure of the worth of an individual clutch for an individual of a given species. Species with a high brood value tend to invest more into their individual clutches as they represent a larger fraction of their total reproductive success, while species with a low brood value are under less pressure to succeed with every individual clutch, and may not invest as much care (Bókony et al. 2009).

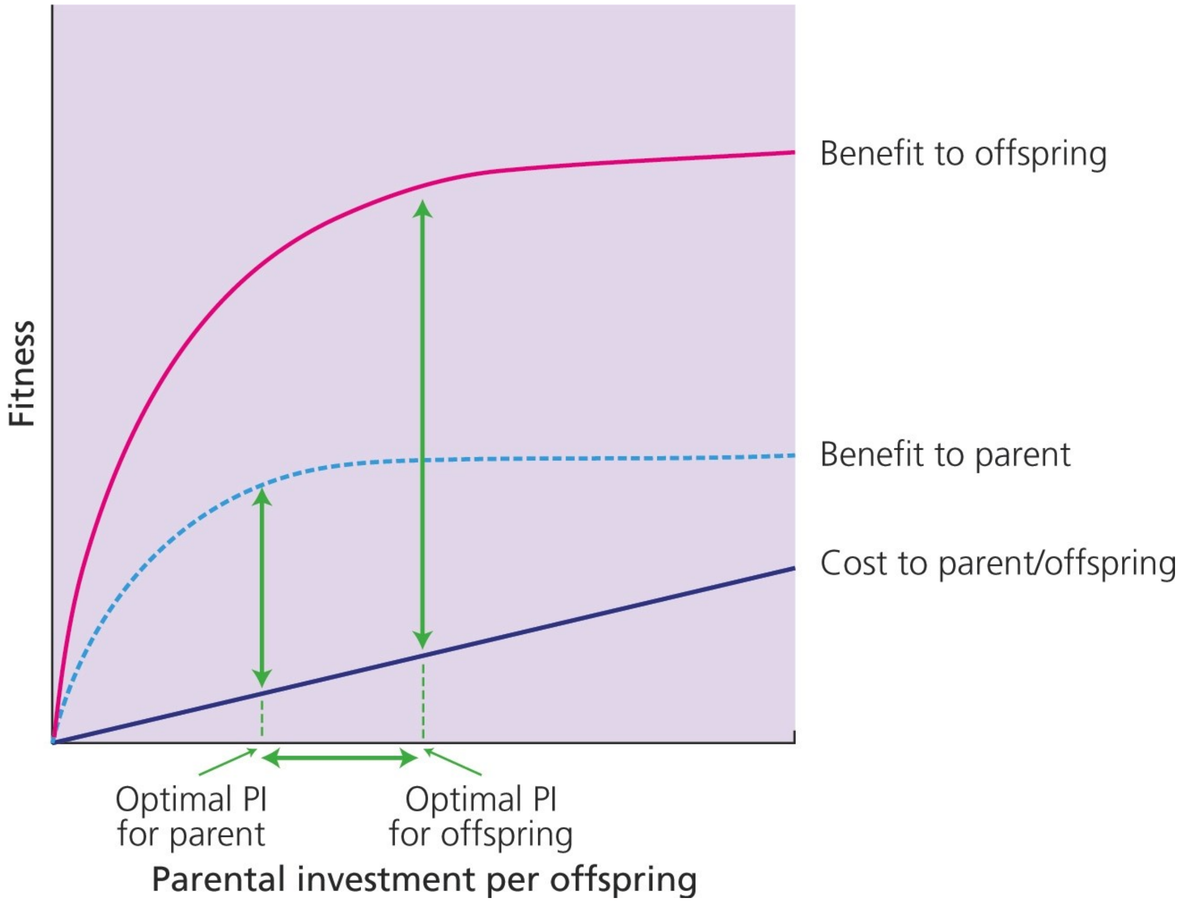

FIGURE 1.1. Parent-offspring conflict schematized, with curves corresponding to the fitness benefits for the offspring (red), parent (dotted light blue) and the cost to parent and offspring (dark blue), depending on parental investment per offspring. The conflict between parent and offspring is illustrated by the difference between the optimal investment for maximal offspring fitness (rightmost green arrow) and for maximal parental fitness (leftmost green arrow). Taken from Davies et al. (2012).

<u>Clutch abandonment</u>

As parental care incurs costs that can be deleterious to the parents, there is a limit to the care parents can invest in a clutch. If the reproductive value of a brood is outweighed by the cost of providing for the brood, parents may abandon their offspring in favour of investing towards a potentially more successful future reproductive output and ultimately maximize their lifetime reproductive success (Winkler 1991; Wiggins et al. 1994; Zuckerman et al. 2014).

In taxa that lay clutches, this is referred to as clutch abandonment. Clutch abandonment has been widely reported in a variety of taxa such as fish, mammals, insects and birds (Rothstein 1975; Tait 1980; Hiraiwa-Hasegawa and Hasegawa 1988; Zink 2003; Zuckerman et al. 2014; Klug and Bonsall 2019). Clutch abandonment can either happen because of external or environmental factors (*e.g.* inclement weather) or due to the health of the individual jeopardizing the individual's ability to pursue nesting effort. For instance, negative weather conditions are known to increase the likelihood of clutch abandonment (Thierry et al. 2013), and species of penguins abandon their clutch when reaching poor body condition, a reaction that was associated with increased baseline corticosterone (CORT), the main stress hormone in birds (Siegel 1980; Groscolas et al. 2008; Spée et al. 2010, 2011). Overall clutch abandonment is an important mechanism in certain species to respond to unexpected challenges of reproduction.

Clutch abandonment is adaptive or not depending on the cost it has on the overall reproductive success of the parent (Lessells 1993). In challenging conditions, clutch abandonment can be an adaptive response to lay a new clutch in potentially more auspicious conditions (Wiggins et al. 1994; Bókony et al. 2009; Zuckerman et al. 2014). However, clutch abandonment involves a trade-off between accepting an ongoing clutch of reduced fitness and investing in a new clutch (Sealy 1995). On one hand, reproductive success may be salvaged by beginning a new clutch, but on the other hand, expending more resources incurs a significantly higher cost to the parents, which may be challenging late in the season when food resources are diminished or if their body condition is weakened (Wiggins et al. 1994; Guigueno and Sealy 2010). As a season advances, individuals will be under more pressure to bring a clutch to completion as the window for a new breeding effort recedes (Guigueno and Sealy 2010). In summary clutch abandonment may lead to trade-offs, resulting in a variable response to environmental stressors from individuals.

Brood parasitism

Brood parasitism is a reproductive strategy whereby an individual lays its clutches in the nests of another individual, delegating parental care to this other species (Davies 2000). Brood parasitism is a strong model to study questions of parental investment and trade-offs associated with parental care and clutch abandonment (Thorogood et al. 2019). Obligate brood parasites rely exclusively on host species for offspring rearing (Davies 2000; Feeney et al. 2014; Soler 2017), and are found among a variety of taxa including invertebrates, reptiles, fish and birds (Thorogood et al. 2019). About 1% of all bird species are obligate avian brood parasites, and over 10% of bird species are parasitized globally (Davies 2000; Feeney et al. 2014; Soler 2017).

Brood parasitism reduces or nullifies the host's reproductive success (Soler et al. 1995; Kilner 2004; Medina and Langmore 2015), and has led to the evolution of antiparasitic responses in some host species (Davies 2000; Peer et al. 2005).

Clutch abandonment as an antiparasitic response

Antiparasitic defences may occur prior to the insertion of the parasitic egg (front-line defences), while the parasitic egg is inserted within the host nest (egg-stage defences), or when the parasite has hatched within the host nest and hatchlings are directly competing (nestling-stage defences) (Davies 2000; Abolins-Abols and Hauber 2018; Campobello and Sealy 2018). In birds, at the egg-laying stage the host may evolve one or more types of egg rejection behaviour; egg ejection (grasp or puncture), or clutch abandonment via either egg burial of the clutch under additional layers of nesting material for a new clutch to be laid on top in the same nesting site (a less costly form of clutch abandonment) or nest desertion (a more costly form of clutch abandonment) (Clark and Robertson 1981; Sealy 1995; Moskát and Honza 2002; Peer et al. 2005; Guigueno and Sealy 2009). Clutch abandonment is a distinct antiparasitic response from egg ejection. Indeed, egg ejection (i.e., physical removal of the egg via grasping or puncturing it with the bill) enables species capable of it to avoid the costs of clutch abandonment, where all investment in a given clutch is lost and the host must invest in laying a new clutch (Servedio and Hauber 2006). However, the ability to eject is only present in certain species, because it is partly dependent on morphological constraints (Servedio and Hauber 2006; Rasmussen et al. 2010; Soler et al. 2015). Clutch abandonment is an antiparasitic response that happens either through nest desertion and renesting at a new site, or egg burial for a new clutch to be laid on a superimposed nest. Clutch abandonment is comparatively costlier than egg ejection in terms of energy and time invested (Clark and Robertson 1981; Sealy 1995; Hosoi and Rothstein 2000; Moskát and Honza 2002; Guigueno and Sealy 2009; Mark and Rubenstein 2013; Soler et al. 2015). Clutch abandonment in response to brood parasitism is not an absolute response, as individuals abandon parasitized clutches only part of the time, contrasting with egg ejection which tends to be either high or low in a given population (Guigueno and Sealy 2012). The inconsistency in enacting antiparasitic defenses is hypothesized to be due to either a maladaptive response caused by insufficient common history with the parasite (Peer and Sealy 2004), resulting in an evolutionary lag (evolutionary lag hypothesis) (Underwood and Sealy 2006), or to an adaptive equilibrium where parasitism acceptance is less costly than abandonment (evolutionary equilibrium hypothesis) (Medina and Langmore 2016; Avilés 2018). Another hypothesis is that parasites retaliate against hosts that defend themselves from parasitism by destroying their clutch, promoting some degree of parasitism acceptation from the host species (Chakra et al. 2014, 2016). The factors and mechanisms underlying variation in antiparasitic clutch abandonment response are largely unexplored but constitute a developing field (Avilés 2018; Abolins-Abols and Hauber 2018).

Study species

This thesis focuses on a group of American avian obligate brood parasites, *Molothrus sp*, commonly called cowbirds, and their hosts. There are five parasitic cowbird species; the brown-headed cowbird (*Molothrus ater*), shiny cowbird (*Molothrus bonariensis*), bronzed cowbird (*Molothrus aeneus*), giant cowbird (*Molothrus oryzivorus*), and screaming cowbird (*Molthrus rufoaxillaris*), whose combined ranges cover the entirety of North and South America, and cumulatively account for more than 200 species as hosts. Cowbirds are notable among obligate brood parasites in that the parasite nestlings do not outright remove the host's own egg and nestlings, either through ejection or hatchling killing, as seen in the extensively studied common cuckoo (*Cuculus canorus*) (Davies 2000, 2015). As a result, the cowbird and host nestlings will often be reared together in the same nest. In many species, the cowbird will outcompete the host's offspring due to their shorter incubation period, increased size and growth rate, leading to a suppression of the host's reproductive success (Briskie and Sealy 1990; Hosoi and Rothstein 2000). In larger species, the host offspring can better compete against cowbirds and have an increased chance of having at least some nestlings survive. Cowbirds are historically prairie species, whose ranges have expanded following habitat modification such as deforestation and agriculture to parasitize more regularly species that previously had limited contact with them, leading cowbirds to parasitize naïve populations and species (Mayfield 1965; Cruz et al. 1985; Smith et al. 2000; Kostecke et al. 2004; Chace et al. 2005).

While analyzing the behaviour of cowbirds for a large number of hosts is useful, it is also useful to investigate how a specific species responds to parasitism. As part of Chapter 3 of this thesis, a special attention is also given to the cowbird host the yellow warbler (*Setophaga petechia*), a common North American passerine that is frequently parasitized by the brown-headed cowbird and is suitable to study clutch abandonment in the context of brood parasitism. They are the only host of the cowbird known to regularly reject parasitized clutches by two forms of clutch abandonment: nest desertion and egg burial (Clark and Robertson 1981; Sealy 1995; Mico 1998; Hauber et al. 2006; Guigueno and Sealy 2012). Warblers are more likely to bury or desert parasitized clutches early in the breeding season when there is time to re-nest (Guigueno and Sealy 2010). On average, about 35% of female yellow warblers bury cowbird eggs, sometimes with their own eggs if any were laid, and attempt to renest at the same site. Another 12% of females desert the nest entirely and renest at a new site (Sealy 1995). If parasitism occurs early in the laying period, roughly 50% of females bury (Clark and Robertson 1981). However, warblers rarely desert when parasitized during incubation, possibly due to significant costs related to time lost and building an entirely new nest (Guigueno and Sealy 2009). Due to these high costs of clutch abandonment, warblers may rely on multiple stimuli when making their decision, such as interaction with the adult parasite at the nest (Guigueno and Sealy 2011).

Factors influencing clutch abandonment

Life history factors

Several factors are thought to come into play in rejection behaviour in the context of parasitism. As brood value is an integrated quantification of lifetime reproductive success (Bókony et al. 2009), it can be expected to be inversely related to clutch abandonment, as parental care would be modulated depending on the importance of a given clutch for the species. In the context of parasitism, the cost of abandoning a clutch in a high brood value species may outweigh the benefits of accepting it (Avilés et al. 2005).

Habitat is also an important factor in parasite-host interactions. In line with the evolutionary lag hypothesis, host species that have a longer evolutionary history with parasites have more time to adapt at the evolutionary scale and may exhibit stronger, more fine-tuned responses to parasitism (Rothstein 1975). For instance, due to their range expansion, cowbirds encounter species that do not have the same evolutionary history of prairie species (Smith et al. 2000). These species are often more vulnerable, as they lack the experience and adaptations to respond to being parasitized. Similarly, some habitats are visited less often by cowbirds looking for hosts. Cowbirds are open-habitat birds while forest hosts tend to be more sheltered, leading forest species to encounter parasitism less often than open habitat species (Smith et al. 2000). However habitat fragmentation related to agriculture and urbanization increases the presence of cowbirds in forest habitats (Morrison and Hahn 2002). Overall, habitat differences can lead to differences in response to parasitism and in clutch abandonment.

Another factor influencing the response to parasitism is host sensitivity. The fitness cost of parasitism differs from one species to another (Hosoi and Rothstein 2000). While some species can successfully rear their own young alongside a cowbird nestling, others experience complete clutch failure (Davies 2000). This is predicted by certain parameters like incubation time and the host’s size relative to the cowbird (Hosoi and Rothstein 2000). Cowbirds have a short incubation time that gives them an advantage in hatching date against the hatchlings of the host species (Briskie and Sealy 1990). As a result, hosts with longer incubation times are more vulnerable to cowbirds (Marvil and Cruz 1989). Similarly, hosts that are significantly smaller than their parasitic cowbird will be more negatively impacted by having to rear a comparatively large cowbird chick, compromising the survival of their own offspring (Hosoi and Rothstein 2000). This variation in fitness cost may be important for predicting variations in clutch abandonment rates, as host species with a lower sensitivity to parasitism will have a greater likelihood to retain some of their fitness when accepting a parasitized clutch (Medina and Langmore 2016).

Finally, migratory behaviour may be involved in clutch abandonment differences. Migratory behaviour poses time, energy and survival constraints on individuals, and migratory birds will often have a comparatively shorter breeding season and fewer breeding opportunities (Zink 2011) which may be reflected with changes in brood value and clutch abandonment rates.

Mechanistic factors

Hormones play a crucial role in modulating behaviour (Tata 2005) and mediate many behavioural adjustments in birds, including antiparasitic responses (Ruiz-Raya et al. 2018;

Abolins-Abols and Hauber 2020b). Specifically, the glucocorticoid hormone, corticosterone, is a principal mediator in regulating behaviours of birds by increasing in response to environmental stressors (Siegel 1980). Elevated CORT can trigger behavioural changes that promote individual survival (Wingfield 2003; Angelier et al. 2009; Crossin et al. 2012), such as increasing vigilance while decreasing expression of reproduction-related behaviours (Wingfield et al. 1998; Wingfield 2003; Schoenle et al. 2017). Therefore, CORT is an ideal candidate to study a host's responses to brood parasitism (Bókony et al. 2009). Simply the presence of a parasitic egg in the nest may increase the host's baseline CORT levels (Ibáñez-Álamo et al. 2012; Mark and Rubenstein 2013; Schoenle et al. 2017; Abolins-Abols and Hauber 2020a) and recent evidence suggests that antiparasitic defenses may be partly mediated by CORT (Abolins-Abols and Hauber 2020a). Abolins-Abols and Hauber (2020a) experimentally manipulated CORT levels in egg grasp-ejecting American robins (*Turdus migratorius*) and found that parasitized clutches were more likely to be accepted when potential increases in baseline CORT levels were inhibited. As baseline CORT represents the conditions under which a bird was under when sampled (Ruiz-Raya et al. 2018), its changes in response to experimental parasitism can provide further insight into the mechanisms mediating a host's response, including clutch abandonment (Landys et al. 2006; Scharf et al. 2021). Feather CORT can thus be used as a biomarker for an integrated measure of CORT secretion representing stress levels over a long period, rather than to a specific environmental stressor revealed by measures of baseline CORT from plasma (Fairhurst et al. 2013). Hence, feather CORT, representing general stress conditions from a different phase of the annual cycle, may reflect how individuals respond to current reproductive decisions due to the integration of carry-over effects (Martínez-Padilla et al. 2013; Hansen et al. 2016). This idea is analogous to the developmental stress hypothesis, which predicts that stress early in life can affect behaviour and stress responses later in life, such as a major reproductive decision like abandoning a clutch (Nowicki et al. 2002; Spencer and MacDougall-Shackleton 2011).

While questions on hormonal mechanisms are a more recent approach in addressing egg rejection by brood-parasitic hosts, a longer-standing perspective is the study of visual differences in colour or maculation between host and parasitic eggs (Rothstein 1974, 1990; Brooke and Davies 1988; Lyon 2003; Manna et al. 2017; Gómez et al. 2021). The ability of a parent to successfully recognize their own eggs, or a discordant egg within their clutch, and reject a parasitic egg should be adaptive as the host would no longer incur the costs associated with brood parasitism (Hauber et al. 2019; Ducay et al. 2021). Visual signal recognition ability may largely rely on the extent of differences in maculation between parasitic and host eggs (Rothstein 1982; Lahti 2006; Cassey et al. 2008; Spottiswoode and Stevens 2010; Honza and Cherry 2017; Hanley et al. 2019). Cuckoo egg rejection increases with differences in appearance, like maculation percentage and spot size, between cuckoo and host eggs (Davies 2000). Thus, this ability to recognize a foreign egg may initiate rejection via egg ejection, but the role of differences in egg visual signals in the context of clutch abandonment is less clear (Davies and Brooke 1989; Langmore et al. 2005; Spottiswoode and Stevens 2010). In addition to egg visual signals stimulating rejection, they may also affect baseline CORT levels. Indeed, Eurasian blackbirds have higher baseline CORT levels when parasitized with non-mimetic eggs (Ruiz-Raya et al. 2018). Hence, clutch abandonment may then be mediated by increases in baseline CORT initiated by the extent of maculation characteristic differences between parasitic eggs and their own.

Thesis overview

In this thesis, I will explore the parental care decisions by investigating the importance of factors underlying clutch abandonment rates differences in hosts of the *Molothrus* genus of avian obligate brood parasites. Two approaches are covered: one from a life history perspective and one from a mechanistic perspective.

In Chapter 2, I conducted a phylogenetically controlled meta-analysis of the existing literature on abandonment rates in commonly parasitized cowbird hosts and cross-reference these data with life history data on each species' brood value, breeding habitat, host sensitivity and migratory status. Because brood value represents the importance of a clutch to lifetime reproductive success, it can be expected that clutch abandonment rates would be negatively related to brood value (Bókony et al. 2009). Furthermore, differences in habitat associated with different levels of cowbird-host interaction may be associated with brood value and impact clutch abandonment rates (Hosoi and Rothstein 2000). High host sensitivity to parasitism can also be associated with increased clutch abandonment rates (Hosoi and Rothstein 2000). I found that brood value predicted clutch abandonment under specific conditions, in unparasitized nests from species from lower cowbird-host interaction environments. The absence of relationship in the presence of parasitism and in more frequently parasitized species may suggest the magnitude of the cost of parasitism overrides brood value. Higher host sensitivity decreased clutch abandonment, but without a direct link to brood value. Overall, these life history traits constitute important variables to consider to explain differences in clutch abandonment.

While a life history point of view seeks to explain differences among species, it is similarly interesting to explore how these processes may be mediated at the level of the individual. In this spirit, Chapter 3 takes a more mechanistic approach, investigating the hormonal and visual processes involved in mediating the decision to abandon, by focusing on the response to experimental parasitism of one host species, the yellow warbler. Levels of the stress hormone CORT are key in mediating clutch abandonment in birds (Abolins-Abols and Hauber 2020a). Egg visual differences are a well-studied aspect of parasitism detection and response (Gómez et al. 2021). Both may interact with each other in mediating clutch abandonment. I experimentally parasitized nests of warblers with model cowbird eggs to investigate the relationship between CORT, egg visual characteristics, and clutch abandonment. I found evidence that, compared to controls, individuals that abandonment experimentally parasitized clutches had higher circulating CORT, while individuals that accepted did not. Visual characteristics did not interact with CORT to mediate abandonment. The combination of results provides a comprehensive understanding of the effects of hormones and egg visual signals in the context of clutch abandonment in brood parasitism specifically, and of parental care more broadly.

Overall, by covering two angles of study this thesis provides evidence for the importance of life history traits such as brood value, cowbird-host interactions, and plasma CORT levels in mediating differences in clutch abandonment response, and offers a varied exploration of clutch abandonment in the context of brood parasitism.

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CHAPTER 2:

A META-ANALYSIS OF LIFE HISTORY TRAITS AFFECTING CLUTCH ABANDONMENT IN COWBIRD HOSTS: PARASITISM OVERRIDES BROOD VALUE IN ENVIRONMENTS WITH ELEVATED PARASITE-HOST INTERACTIONS

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Abstract

Parental care is a fundamental principle of behavioural ecology. If the cost of investing in the current reproductive effort outweighs the benefits, parents may choose to invest in future opportunities and abandon the current investment. Obligate brood parasitism, a reproductive strategy where a parasitic species lays its egg in the nest of another species, can incur negative fitness costs on host species and can serve as a trigger for clutch abandonment in species that do not have the ability to eject the parasitic egg. However, clutch abandonment is also considered a more general response to factors other than brood parasitism such as inclement weather or predation. No study has measured clutch abandonment in the context of brood parasitism alongside these other generalized factors in multiple parasitic species. We conducted a phylogenetically controlled meta-analysis covering 85 hosts of 3 cowbird species (*Molothrus* sp.) in the Americas. Specifically, we compiled records of naturally parasitized and unparasitized abandonment rates of host species. We then compared these rates to life history traits: brood value, small scale habitat (forest vs open) and large-scale habitat (prairie vs non-prairie) to model cowbird encounter rates, mean body weight, and migratory behaviour. Overall, parasitism increased clutch abandonment. This increase was stronger in open habitats where parasites and hosts are more likely to interact. Brood value predicted clutch abandonment in habitats with less cowbird-host interaction, specifically forested and non-prairie habitats, which are measures of small- and large-scale habitat, respectively. Larger hosts were less likely to abandon regardless of parasitism. Migratory species, which generally have a higher brood value, were less likely to abandon when parasitized. These results suggest that parasitism affects clutch abandonment in many ways. The effect of parasitism swamps out the effect of brood value 1) in species with more experience with the parasite (open and prairie) and 2) in species in general when parasitized. Our study emphasizes the importance of taking a multifactorial approach to highlight how multiple parameters interact with each other to explain variation in clutch abandonment among species.

Keywords: avian brood parasitism, parental investment, life-history theory, *Molothrus* sp., nest desertion, egg burial

Introduction

Fitness trade-offs are based on the concept that individuals have a limited number of available resources to allocate to competing functions or structures (Santos and Nakagawa 2012; Braendle et al. 2013). The cost of reproduction and parental care is one of the most widely studied fitness trade-offs and is fundamental in understanding life-history evolution (Linden and Møller 1989; Nilsson and Svenssonn 1996; Harshman and Zera 2007; Rauset et al. 2015; Richardson et al. 2020). Parental care decisions are based on a trade-off between investing in current reproductive success and future reproductive potential where parents are trying to maximize their total lifetime reproductive output (Remick 1992). Parental care is therefore predicted to be costly because an individual that allocates more resources to a current offspring is expected to suffer from reduced future fecundity or survival (Mark and Rubenstein 2013; Williams 2018). Studies that experimentally manipulate brood sizes show that increasing the number of offspring often results in future fitness costs for the parents like reduced body condition and lifespan (Velando and Alonso-Alvarez 2003; Santos and Nakagawa 2012). As a result, parental care varies based on a parent's perceived value of a brood (Williams 1966; Gross 2005). If the reproductive value of a brood is outweighed by the cost of providing for the brood, parents may abandon their offspring in favour of investing towards future reproductive output and ultimately maximize their lifetime reproductive success (Zuckerman et al. 2014). Thus, when parental care becomes too costly, parents can opt to abandon a nesting effort and rescue their remaining resources to reinvest them into a potentially more successful future reproductive output (Wiggins et al. 1994). Parental brood abandonment has been widely observed across taxa such as fish, insects, mammals and birds (Rothstein 1975; Tait 1980; Hiraiwa-Hasegawa and Hasegawa 1988; Zuckerman et al. 2014; Klug and Bonsall 2019).

Birds are an ideal system to study clutch abandonment because of their diverse ecological niches and the existence of much associated research (Tobias et al. 2020). Birds tend to lay clutch sizes that maximize their fitness (Tinbergen and Daan 1990) and if the value of the current breeding attempt is reduced, parents must reconsider whether it is worth continuing investing in the current breeding attempt or stop investing completely (Lessells 1993). During a single breeding season, reproductive success may be salvaged by beginning a new clutch, but this decision requires expending more resources and incurs a significantly higher cost to the parents, which can be challenging late in the season when resources are increasingly diminished and body conditions are weaker. There are several cues that can elicit abandonment in birds that vary between and within species such as predation (Lima 2009), harsh weather conditions (Bottitta et al. 2003), and poor body condition (Groscolas et al. 2008; Spée et al. 2010, 2011). For example, waterfowl have a tendency towards offspring abandonment following partial nest predation (Armstrong and Robertson 1988; Ackerman and Eadie 2003). Thus, under these conditions, clutch abandonment can be a response that allows parents to lay a new clutch in potentially better conditions, increasing their reproductive success within a breeding season or across their lifetime (Bókony et al. 2009; Guigueno and Sealy 2010).

Brood parasitism constitutes a strong model to study questions of parental care. About 1% of all bird species are obligate avian brood parasites (approx. 100 sp.), which exclusively lay their eggs in nests of other species (Feeney et al. 2014; Soler 2017). Over 10% of all bird species are parasitized globally (approx. 950 sp., Davies 2000) and these hosts incur a significant fitness cost while also decreasing their reproductive success when having to rear the added parasite offspring (Medina and Langmore 2015). The reproductive cost of caring for a parasite selects for, in some hosts, antiparasitic defence adaptations that minimize the negative fitness consequences of raising a parasite (Feeney et al. 2014). By using brood parasitism as a model, we can study the modulation of parental care using host antiparasitic defences.

A large proportion of host species have evolved diverse antiparasitic responses to being parasitized that allow hosts to preserve their fitness against parasites (Davies 2000; Peer et al. 2005). These defences may occur prior to the insertion of the parasitic egg (front-line defences), while the parasitic egg is inserted within the host nest (egg-stage defences), or when the parasite has hatched within the host nest as parents may favour parental care towards their own offspring (nestling-stage defences) (Davies 2000; Schuetz 2005, Abolins-Abols and Hauber 2018; Campobello and Sealy 2018). In the present study we focus on egg-stage defences that hosts may use when having a parasitized egg placed within their nest. Firstly, grasping parasitic eggs and ejecting them from the nest has low costs on the host and enables a host species to avoid abandoning a clutch entirely (Servedio and Hauber 2006). However, the ability to eject is only present in certain host species because it is partly dependent on morphological constraints such as gape-size limitations (Rasmussen et al. 2010; Guigueno and Sealy 2011; Soler et al. 2015). Some species also rely on puncture ejection, where the parasitic egg is impaled and moved out of the nest (Sealy 1996). This strategy also has a lower fitness cost compared to clutch abandonment, although it is hypothesized to bring a higher risk of damage to the host's eggs, especially in smaller hosts (Sealy 1996; Underwood and Sealy 2006). Clutch abandonment as an antiparasitic response, through either nest desertion and renesting at a new site or egg burial for a new clutch to be laid on a superimposed nest, is comparatively costlier than egg ejection in terms of energy invested and time (Clark and Robertson 1981; Sealy 1995; Hosoi and Rothstein 2000; Moskát and Honza 2002; Guigueno and Sealy 2009; Mark and Rubenstein 2013; Soler et al. 2015). Within a population, clutch abandonment is more of an intermediate response whereas grasp ejection is either all or none (Guigueno and Sealy 2012). The inconsistency in enacting antiparasitic defenses is hypothesized to be due to either a maladaptive response caused by insufficient common history with the parasite (Peer and Sealy 2004), resulting in an evolutionary lag (evolutionary lag hypothesis) (Underwood and Sealy 2006), or to an adaptive equilibrium where parasitism acceptation is less costly than abandonment (evolutionary equilibrium hypothesis) (Krüger 2011; Kilner and Langmore 2011; Medina and Langmore 2016; Avilés 2018). The justification for the diversity of occurrence of brood parasitism and associated host response remains unclear (Feeney et al. 2013), and some differences are associated with different brood parasitic clades. The cowbird (*Molothrus* sp.) system in the Americas distinguishes itself from the common cuckoo system in Eurasia (Friedmann 1963; Lanyon and Omland 1999; Feeney et al. 2014). Unlike the extensively studied common cuckoos (*Cuculus canorus*), cowbird nestlings typically do not evict host eggs or nestlings from the nest, and have a lower fitness cost comparatively (Feeney et al. 2014). Cuckoos are an older parasite group (>5 million years) that are more specialized with subspecies specializing in different host species, whereas cowbirds are a comparatively more recent parasitic group (<5 million years) and more generalist (Feeney et al. 2014). The five cowbird species together parasitize over 300 hosts (Lowther 2018). The cues that elicit clutch abandonment through desertion or burial as an antiparasitic response remain unclear and continue to interest researchers (Hosoi and Rothstein 2000; Kosciuch et al. 2006; Manna et al. 2017; Avilés 2018; Abolins-Abols and Hauber 2020).

Clutch abandonment may be influenced by a variety of factors, including brood value. Brood value is defined as the importance of a given clutch relative to the total reproductive effort for a given species. Brood value can be quantified as (Bókony et al. 2009; Sol et al. 2016):

$$\log\left(\frac{clutch\ size}{clutch\ size\ \times broods\ per\ year\ \times maximum\ reproductive\ lifespan}\right)$$

By using this equation, we obtain an integrated measure of brood value. Species with a high brood value tend to invest more into their individual clutches as they represent more of their total reproductive success, while species with a low brood value are under less pressure to succeed with every individual clutch, and may not invest as much care (Bókony et al. 2009). In the context of parasitism, the cost of abandoning a clutch in a high brood value species may outweigh the benefits of accepting it (Avilés et al. 2005).

An important factor to consider in antiparasitic responses is the habitat, which can affect the degree of parasite-host interaction. In line with the evolutionary lag hypothesis, hosts species that have a longer evolutionary history with cowbirds have more time to adapt at the evolutionary scale and may exhibit stronger, more fine-tuned responses to parasitism (Rothstein 1975). We can characterize a difference in the frequency of cooccurrence of the parasite and host both at a large habitat scale in terms of species range, and at a small habitat scale in terms of breeding habitat for this species. At the large scale, *M. ater* (Mayfield 1965)*, M. bonariensis* (Cruz et al. 1985) and *M. aeneus* (Kostecke et al. 2004) are historically prairie species, whose ranges have expanded beyond the prairies following habitat modification such as deforestation and agriculture (Mayfield 1965; Smith et al. 2000; Chace et al. 2005). As a result, cowbirds come into contact with species that do not have the same evolutionary history as prairie species. These species are often more vulnerable, as they lack the experience and adaptations to respond to being parasitized. At the small scale, some habitats are visited less often by cowbirds looking for hosts. Cowbirds are open-habitat birds and primarily search for hosts in these habitats (Mayfield 1965). By comparison, forest hosts tend to be more sheltered, leading forest species to encounter parasitism less often than open habitat species (Smith et al. 2000), although habitat fragmentation related to agriculture and urbanization increases the presence of cowbirds in forest habitats (Morrison and Hahn 2002). Overall, small scale and large-scale habitat differences can lead to differences in response to parasitism.

Another factor influencing the response to parasitism is host sensitivity. The fitness cost of parasitism differs from one species to another (Hosoi and Rothstein 2000). While some species can successfully rear their own young alongside a cowbird nestling, others experience complete clutch failure. This is predicted by certain parameters like incubation time and the host's size relative to the cowbird (Hosoi and Rothstein 2000). Cowbirds have a short incubation time that gives them an advantage in hatching date against the hatchlings of the host species (Briskie and Sealy 1990). As a result, hosts with longer incubation times are more vulnerable to cowbirds (Marvil and Cruz 1989). Similarly, hosts that are significantly smaller than their parasitic cowbird will be more negatively impacted by having to rear a comparatively large cowbird chick, compromising the survival of their own offspring (Hosoi and Rothstein 2000). This variation in fitness cost may be important for predicting variations in clutch abandonment rates, as host species with a lower sensitivity to parasitism will have a greater likelihood to retain some of their fitness when accepting a parasitized clutch (Medina and Langmore 2016).

Finally, migratory behaviour may be involved in clutch abandonment differences. Migratory behaviour poses time, energy and survival constraints on individuals, and migratory birds will often have a comparatively shorter breeding season and fewer breeding opportunities (Zink 2011), which may be reflected with changes in brood value and clutch abandonment rates.

Our overall goal was to examine several factors that may affect clutch abandonment in naturally parasitized cowbird hosts, using data from a large number of species. We conducted a meta-analysis using the existing literature on cowbird hosts to conduct a phylogenetic analysis. We hypothesized that brood value, habitat at large (prairie vs non-prairie) and small (open vs forested habitat) spatial scales, host sensitivity proxies (incubation time and mean body mass) and migratory behaviour would have an influence on clutch abandonment in the context of naturally parasitized and unparasitized nests. Specifically, we predict that clutch abandonment rates will be: 1) negatively associated with brood value, 2) positively associated with habitats with increased cowbird-host interaction at the large and small scales (prairie and open habitats, respectively) relative to habitats with less interaction (non-prairie and forested habitats), 3) increased in high host sensitivity species (whether measured through longer incubation time or smaller body mass), and 4) reduced in migratory hosts given their reduced reproductive opportunities. Our analysis of data from the published literature allows us to test these predictions, and provides us with a fuller understanding of the relationships between life history traits and brood value in the context of clutch abandonment in brood parasitism specifically, and of parental care more broadly.

Methods

General data collection

We reviewed the literature that presented data on host clutch abandonment after natural parasitism events. Data were extracted from all published sources incorporating studies of breeding passerines that included counts of parasitized nests where desertion or burial occurred after parasitism by a cowbird. The studies included in our metanalysis were those where nests were exclusively naturally parasitized, and we excluded all clutch manipulation studies in which a model or natural cowbird egg was added to the nest by an experimenter. Our search included scientific journals, field guides, and previously compiled datasets (see references in supplementary material). Specifically, to be included in the meta-analysis, the following data were required: (1) incidence of natural parasitism occurring to a confirmed host of a cowbird species (Lowther 2018), (2) reporting the number of natural parasitized nests (not total number of cowbird eggs), and (3) reporting the number of clutch abandonments by either nest desertion or egg burial that occurred following a natural parasitism event. Egg burial is not widespread among cowbird hosts but extensively used in some small species like the yellow warbler (Sealy 1995). Studies were excluded if the paper did not specifically state that the cause of desertion was due to a parasitism event and any nests listed as ‘outcome unknown’ were excluded from the study. Nest failures attributed to cowbird parasitism without explaining whether this failure was due to desertion or burial were also excluded from our data set. Similarly, nests whose failure was attributed to other factors, such as weather, predation, parent death or nest destruction were not counted as desertions due to parasitism.

We used Lowther’s (2018) list of hosts of the brood parasitic cowbirds to initially find which host species were true and effective hosts of the cowbirds known to have reared cowbird young successfully. We obtained information on common host species using the Cornell Lab of Ornithology of Birds of the World (Billerman et al. 2020). This database provided us with information on the type of rejection used by a given host (desertion, burial, egg grasp ejection, and/or egg puncture). All grasp ejectors and hosts that egg puncture were excluded from the meta-analysis, so that our database includes only hosts that reject solely through burial or desertion. Since we included only species that do not reject via grasp or puncture ejection, the calculated abandonment rates via desertion or burial were accurate for the population considered. From this, we could extract information on common hosts that desert and/or bury as a type of antiparasitic defense behaviour after being parasitized. Once we had the hosts' general information, we then searched on Google Scholar, Web of Science, and JSTOR for key words: *name of host species, name of Molothrus cowbird species, name of host species-name of Molothrus cowbird species, name of host species-cowbird parasitism, name of host species-abandonment, name of host species-desertion, name of host species-burial*.

Once a study was found that met all criteria, we would then extract rates of clutch abandonment in parasitized and unparasitized (if reported) host nests, number of parasitized nests and unparasitized nests not deserted. The total percentage of all deserted parasitized nests was calculated from the number of deserted parasitized nests over the total number of parasitized nests. This was done as well for the percentage of deserted unparasitized nests in the same way.

These measures were used as estimates of the average species clutch abandonment rate for a given species. We made the assumption that this measure of percentage clutch abandonment was representative of the average for this species with and without parasitism. We assumed that individual variation was taken into account by this average clutch abandonment rate, and that the rate was constant throughout a bird's reproductive life span. The great majority of studies examined did not report individual ages, making it impractical to take age into account in our dataset. A study on a common cowbird host found no have differences in clutch abandonment rates between one year old and older individuals, suggesting that age is not predictor of abandonment rates in the context of parasitism (Sealy 1995). Guigueno and Sealy (2011) also argued that experience with parasitism was not necessarily correlated with age due to the inconsistent occurrence of natural parasitism.

All specifics of the study were included within the database. All life-history information and sensitivity measures on host species were found using the Cornell Lab of Ornithology of Birds of the World (Billerman et al. 2020) including~~,~~ migratory/non-migratory, forest/non-forest, and prairie/non-prairie. We collected mean body weight and average incubation times to serve as proxies to host sensitivity. They were both separated according to a cut-off into “high” and “low” sensitivity yielding a categorical variable. For incubation time, this cut-off was determined by the difference in average incubation time between the host species and the cowbird host. The species was categorized as “low sensitivity” when the host species had a shorter average incubation time and “high sensitivity” when it was not. For body mass, a cut-off of 13g was used to differentiate between large or low sensitivity species and small or high sensitivity species (Hosoi and Rothstein 2000).

Brood value was collected using a database provided by Dr. Daniel Sol from the Centre for Ecological Research and Forestry Applications (CREAF-CSIC) (Sol et al. 2012, 2016). The individual references found within this database were compiled to acquire the variables from which brood value is derived: reproductive longevity and broods per year. We then calculated the brood value from this data using the brood value equation (Sol et al. 2016).

Data analysis

Phylogenetic generalized linear mixed models using Markov chain Monte Carlo (MCMC) analysis with the R package “MCMCglmm” (Hadfield 2010) were used for all analyses (Ducatez et al. 2015, 2020). The models were run for 275001 iterations with a burn-in interval of 25000 to ensure satisfactory convergence. Each model was run to sample 1000 iterations to estimate model parameters. Three random effects were included in all analyses: 1) “phylogeny”, to account for relationships and potential correlations between related species, 2) “species”, to account for repeats of the same species in the dataset, and 3) “population”, to identify parasitized and unparasitized nest samples originating from the same population (i.e. the same study). We assumed that each study represented a distinct population. While this assumption doesn’t hold in cases where more than one study was conducted at the same study site, for the great majority of our data points the variation was captured when assuming that all populations are different, so we considered that this factor could be ignored. The basic aim of all models was to use the rate of clutch abandonment as the response variable against one of either prairie/non-prairie, forest/open, migratory/nonmigratory, and the host sensitivity proxies, incubation time and body mass, as the explanatory variable. For each explanatory variable, the effect of parasitism on clutch abandonment with this explanatory variable was first assessed (Table 2.1). Then, the effect of an explanatory variable on the relationship of brood value and clutch abandonment was assessed, with parasitism still as an additional explanatory variable (Table 2.1). When the triple interaction of parasitism, the explanatory variable and brood value was significant, further post-hoc tests were conducted to investigate how the interaction of the variables changed from treatment to treatment.

Table 2.1. List of all analyses conducted, separated by type of explanatory variables used. "%CA" is the percentage of nests abandoned for a given population. All analyses include "phylogeny", "species" and "population" as explanatory variables, which are omitted from the table for clarity.

Explanatory variables	**Analyses**
Type: Brood value alone	%CA vs Brood Value
Type: Habitat - Prairie/non-prairie - Forest/open	%CA vs Parasitism + Prairie %CA vs Parasitism + Forest %CA vs Brood Value + Parasitism + Prairie %CA vs Brood Value + Parasitism + Forest
Type: Migratory behaviour - Migrant/Non-migrant	%CA vs Parasitism + Migration %CA vs Brood Value + Parasitism + Migration
Type: Host sensitivity to parasitism - Incubation time (high/low) - Body mass (high/low)	%CA vs Parasitism + Incubation time %CA vs Parasitism + Body mass %CA vs Parasitism + Brood Value %CA vs Brood Value + Parasitism + Incubation time %CA vs Brood Value + Parasitism + Body mass
Type: Host sensitivity + habitat - Incubation + Prairie - Body mass + Prairie - Incubation + Forest - Body mass + Forest	%CA vs Brood Value + Parasitism + Incubation time + Prairie %CA vs Brood Value + Parasitism + Body mass + Prairie %CA vs Brood Value + Parasitism + Incubation time + Forest %CA vs Brood Value + Parasitism + Body mass + Forest

Considering nest desertion only or the combination of nest desertion and nest burial provided results that were qualitatively comparable, so for all analyses the combined clutch abandonment rate was used. To test how robust our results were, we also re-ran tests while removing certain cowbird species from the analysis to focus on only one species, brown-headed cowbirds, the most common species in the dataset.

Results

We obtained a total of 314 data points. We collected 219 data points on clutch abandonment rates of parasitized nests from 85 host species of 3 parasitic species: 201 for brown-headed cowbirds, 9 for shiny cowbirds and 9 for bronzed cowbirds. Additionally, thanks to studies reporting abandonment rates in unparasitized nests in addition to parasitized nests, 95 data points were collected on clutch abandonment rates in unparasitized nests from 51 host species: 89 for brown-headed cowbirds, 3 for shiny cowbirds and 3 for bronzed cowbirds.

Brood value on its own had no effect on clutch abandonment (*p*MCMC = 0.549; Figure 2.1). Further analyses were pursued to investigate how this relationship may change according to other variables as shown below.

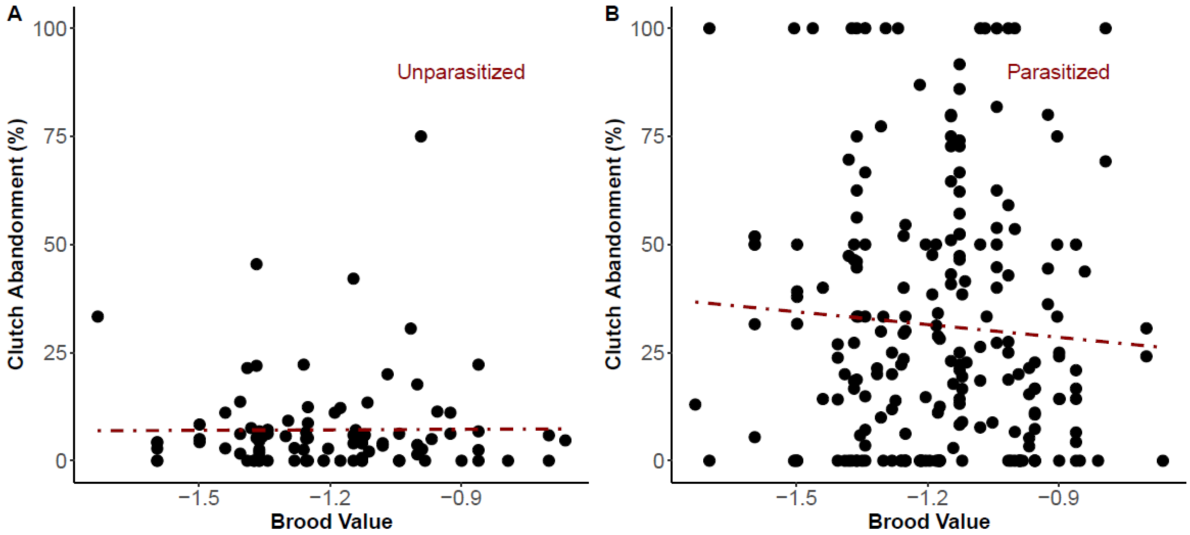

Figure 2.1. Clutch abandonment rate (%) as a function of brood value for unparasitized nests (panel A) and parasitized nests (panel B). The data show no significant trend for either groups.

Large- and small-scale habitat effects

Parasitism was found to increase clutch abandonment in all habitats (*p*MCMC < 0.001). Clutch abandonment rates did not differ between prairie and non-prairie habitats (*p*MCMC = 0.194; Figure 2.2). However, clutch abandonment rates in parasitized nests were higher in open habitats compared to forested habitats (*p*MCMC = 0.006; Figure 2.3). For unparasitized nests, the rate of clutch abandonment did not differ between habitats (prairie/non-prairie and forest/open; Figure 2.2 and 2.3, respectively).

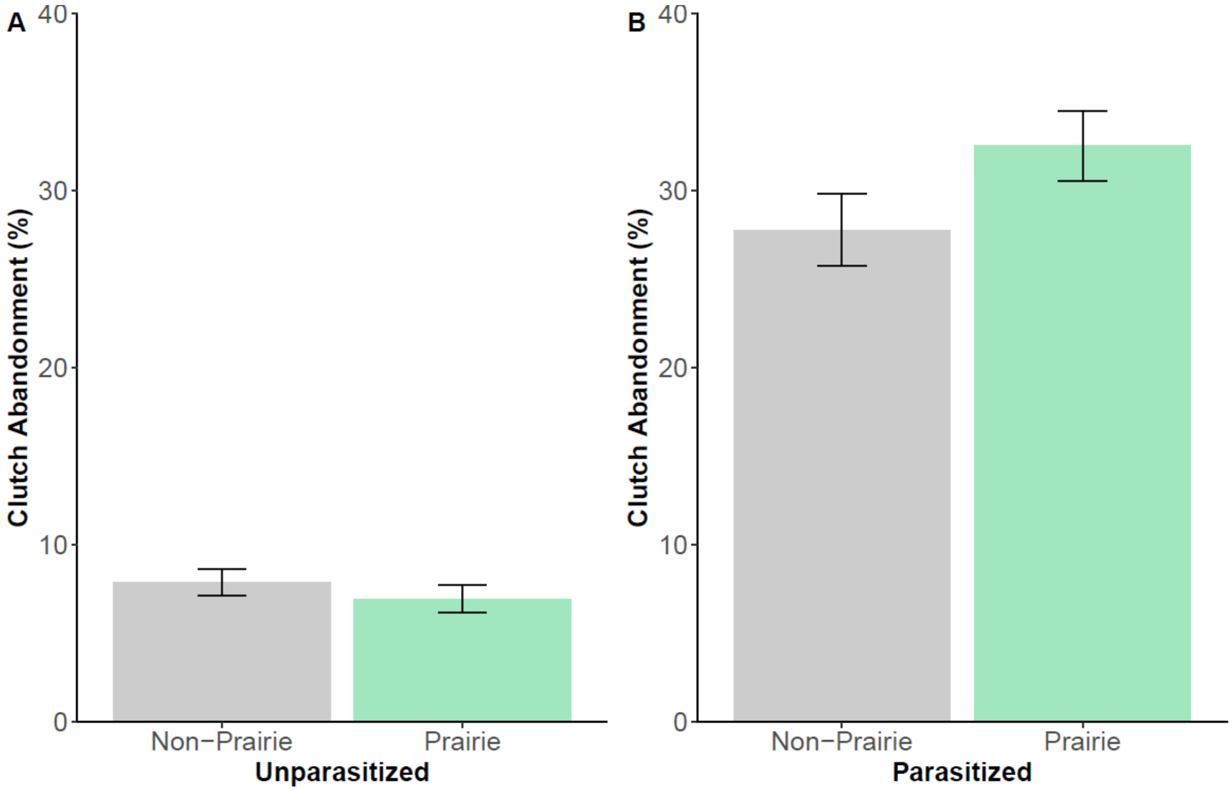

Figure 2.2. Clutch abandonment in function of prairie and non-prairie, for unparasitized (panel A) and parasitized (panel B) nests. Mean + SE. Neither difference was found to be significant. As in Figure 2.1, parasitized nests show significantly higher clutch abandonment, regardless of habitat.

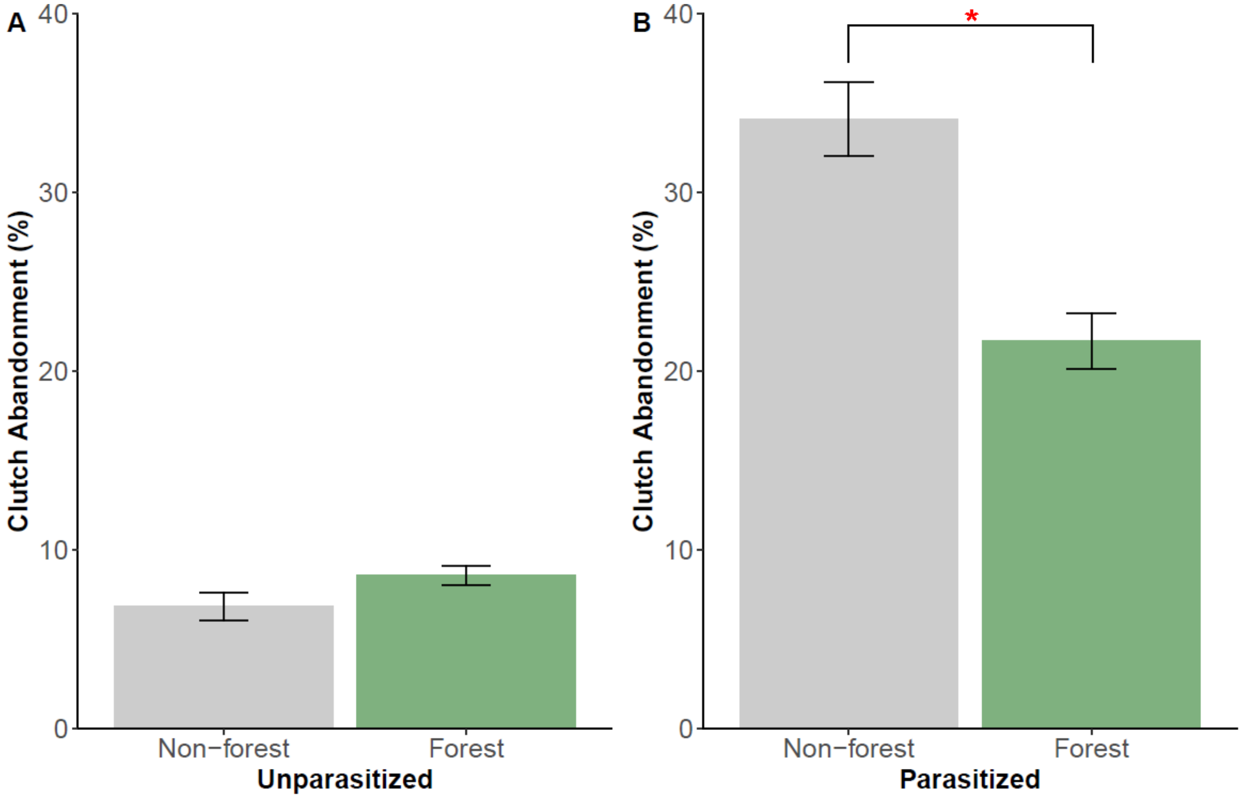

Figure 2.3. Clutch abandonment is shown in function of habitat (non-forest vs forest) for unparasitized (panel A) and parasitized (panel B) nests. Mean + SE. The interaction between parasitism and habitat is significant (*p*MCMC = 0.006). The increase in clutch abandonment in parasitized nests is stronger in non-forest habitats, with no difference between habitats in unparasitized nests (*p*MCMC = 0.230).

The triple interaction of parasitism, brood value and habitat was significant for both prairie/non-prairie (*p*MCMC = 0.040) and open/forest variables (*p*MCMC = 0.002), and a similar pattern was found in both. Increasing brood value predicted decreasing abandonment, but only in unparasitized, non-prairie nests for the prairie variable (*p*MCMC = 0.010; Figure 2.4) and only in unparasitized, forest habitat nests for the forest variable (*p*MCMC = 0.006; Figure 2.5) with no significant relationship in the other subgroups for either variable. Excluding bronzed cowbirds did not change these final results. When excluding shiny cowbirds to focus solely on brown-headed cowbirds, there were no effects of brood value on clutch abandonment.

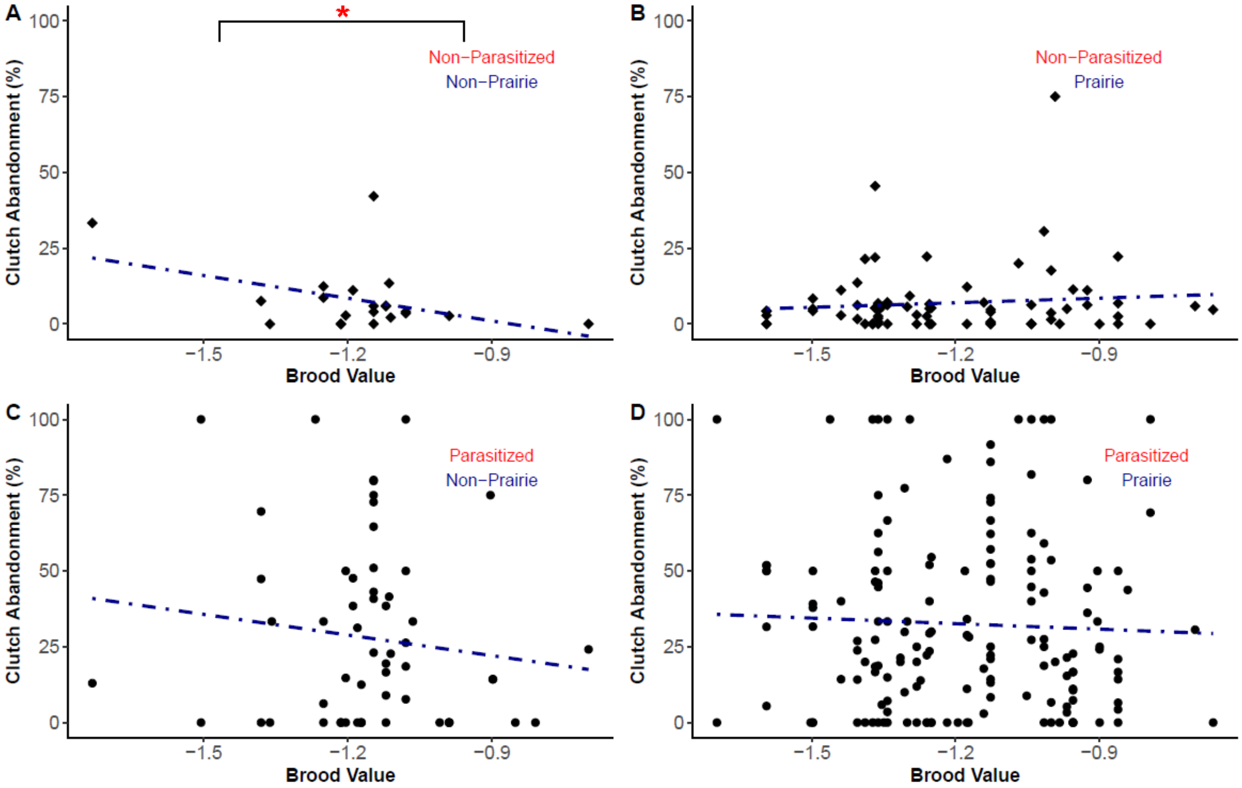

Figure 2.4. Clutch abandonment rate (%) plotted against brood value for different combinations of parasitized (C and D) vs non-parasitized (A and B) nests and of prairie (B and D) vs non-prairie (A and C) habitats. The relationship between brood value and clutch abandonment is significant in unparasitized nests in non-prairie habitat (panel A; pMCMC = 0.010) for which clutch abandonment decreases as brood value increases, consistent with predictions. In all other cases, there is no significant relationship.

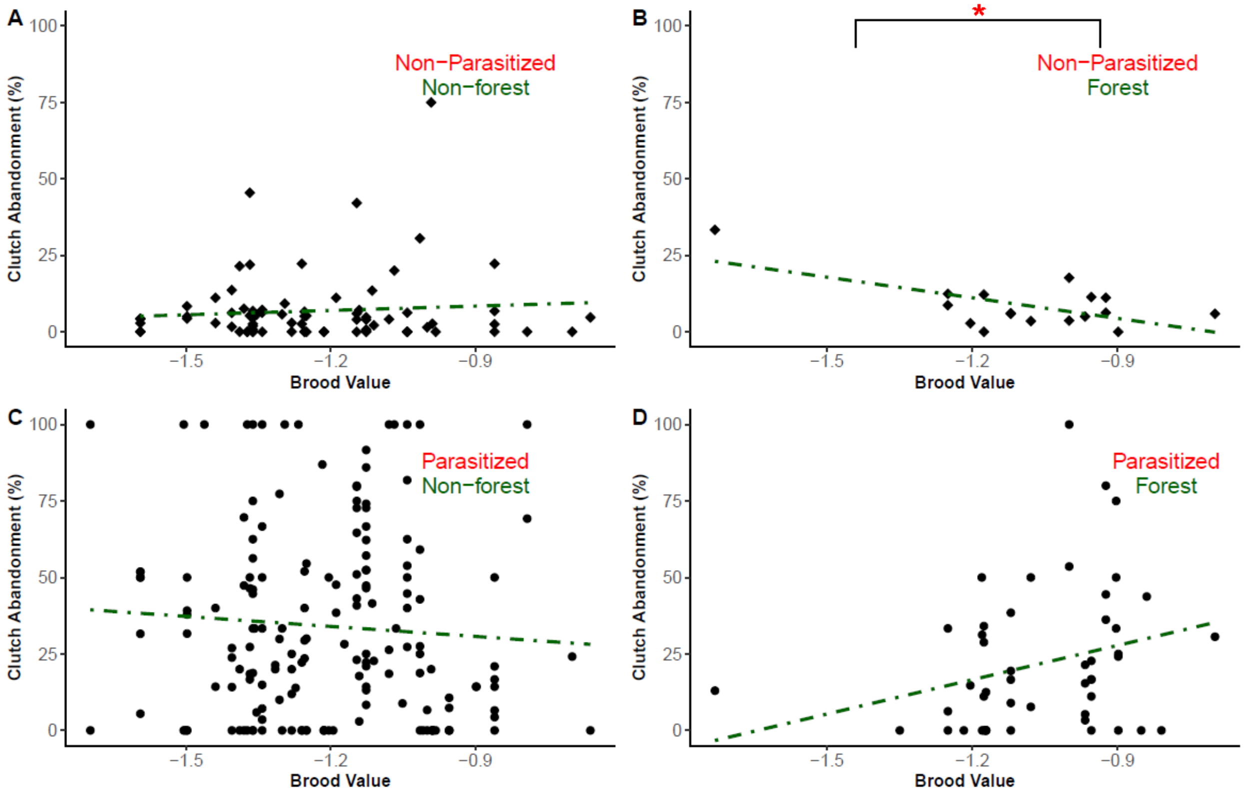

Figure 2.5. Clutch abandonment plotted against brood value for different combinations of parasitized (C and D) vs non-parasitized (A and B) nests and of forest (B and D) vs non-forest (A and C) habitat. The only significant relationship is for unparasitized nests in forest habitats (panel B), for which clutch abandonment decreases as brood value increases (*p*MCMC = 0.006), consistent with predictions. There is no significant trend in other combinations.

Host sensitivity effect

Parasitism sensitivity estimated by incubation duration did not affect nest rejection and response to parasitism (*p*MCMC = 0.206; Figure 2.6). in contrast, parasitism sensitivity estimated by body mass did, as large species were less likely to reject their nest, parasitized or not (pMCMC = 0.004; Figure 2.7). The triple interaction of parasitism, brood value and host sensitivity was not significant for either incubation time (*p*MCMC = 0.106; Figure 2.8) or body mass (*p*MCMC = 0.102; Figure 2.9).

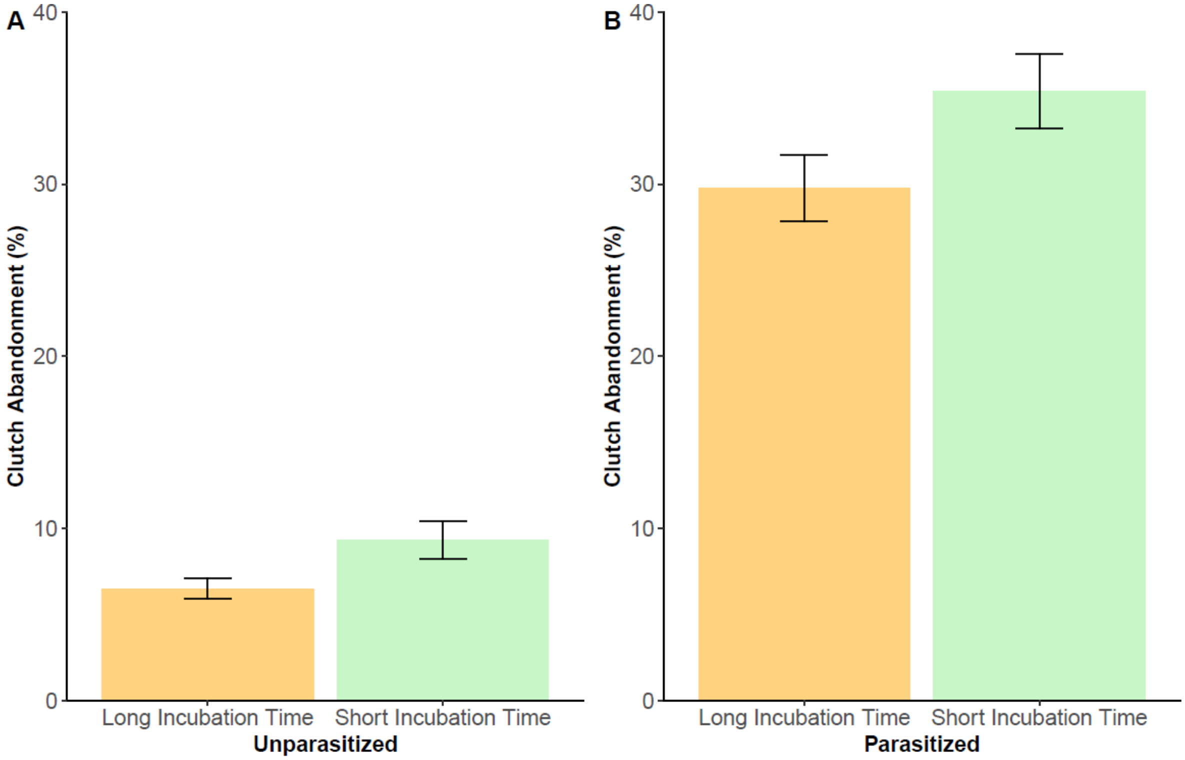

Figure 2.6. Clutch abandonment rates (%) for species with long vs short incubation times for parasitized (panel B) and unparasitized nests (panel A). The interaction between incubation and clutch abandonment is not significant when included (*p*MCMC = 0.587), and the relationship between incubation time and clutch abandonment is not significant (*p*MCMC = 0.206).

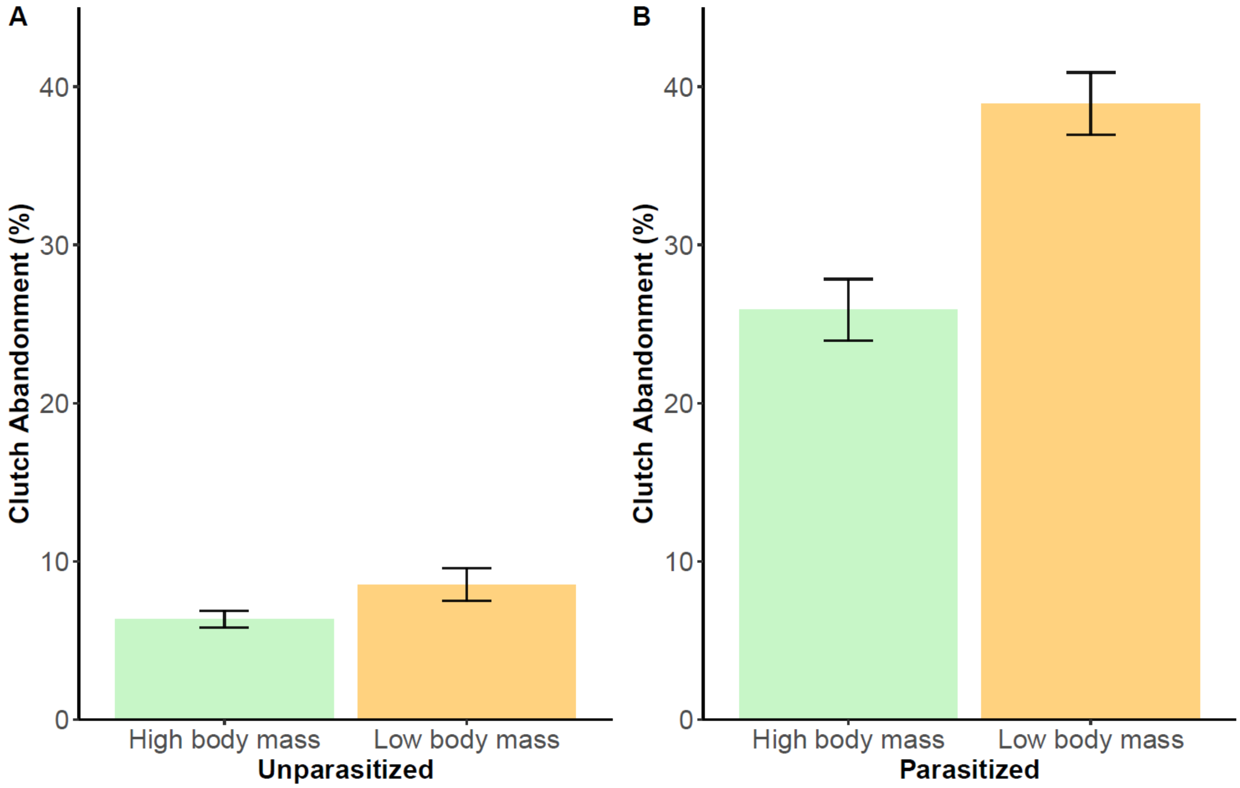

Figure 2.7. Clutch abandonment rates (%) for species of different body masses for parasitized (panel B) and unparasitized (panel A) nests. Mean + SE. Species with low body mass have larger clutch abandonment values (*p*MCMC = 0.004) regardless of parasitism. The interaction between body mass and clutch abandonment was not significant when included in the analysis (*p*MCMC = 0.140).

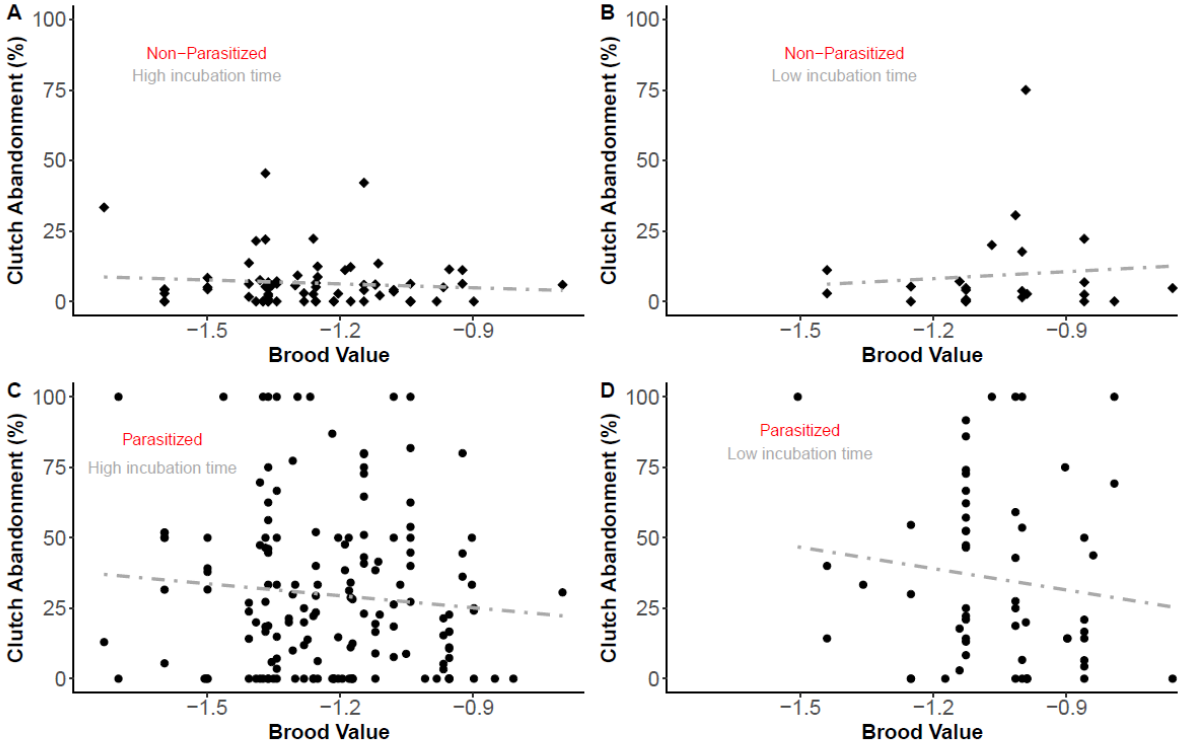

Figure 2.8. Clutch abandonment plotted against brood value for different combinations of parasitized vs non-parasitized nests and of low vs high incubation time. Panel A shows this relationship for non-parasitized nests of high incubation time species, panel B for non-parasitized nests of low incubation time species, panel C for parasitized nests of high incubation time species, and panel D for parasitized nests of low incubation time species. All trends are not significant.

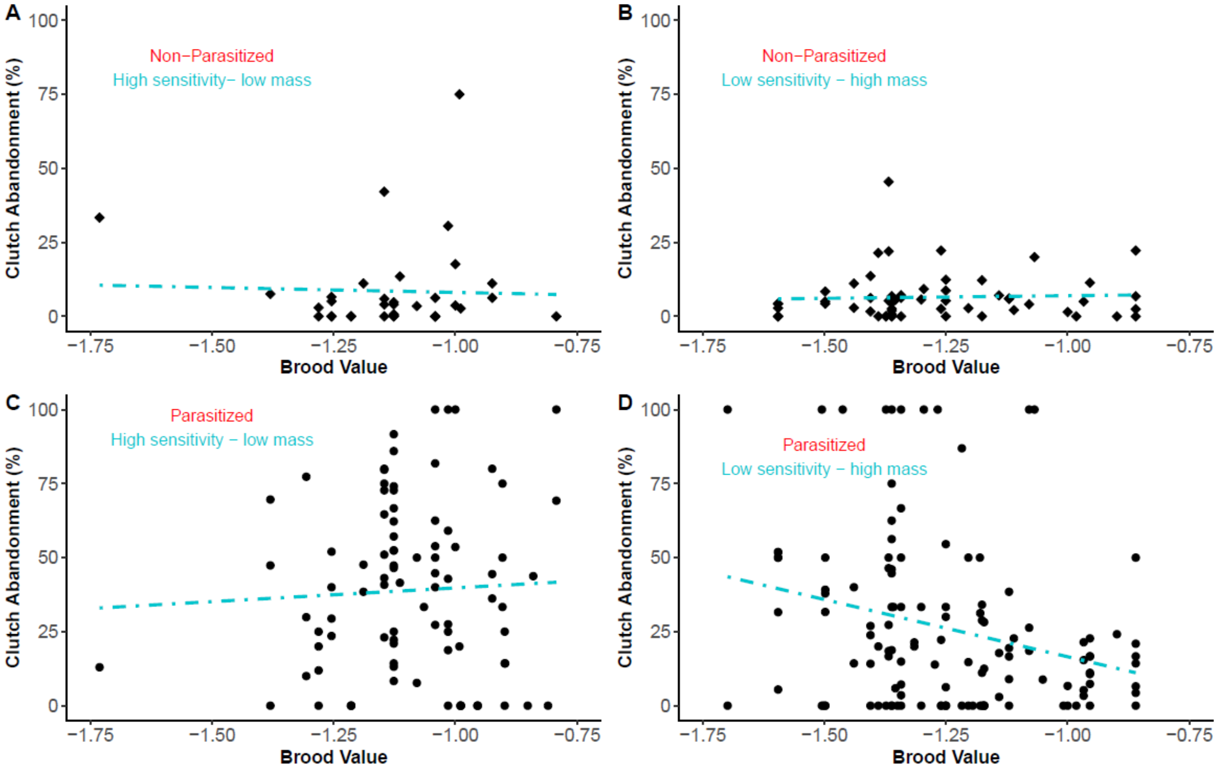

Figure 2.9. Clutch abandonment plotted against brood value for different combinations of parasitized vs non-parasitized nests and of low vs high body mass. Panel A shows this relationship for non-parasitized nests from low body mass species, panel B for non-parasitized nests of high mass species, panel C for parasitized nests of low mass species, and panel D for parasitized nests from high mass species. All trends are not significant.

Combined habitat and sensitivity effect

When considering the combined effect of habitat and host sensitivity on clutch abandonment in a quadruple interaction of parasitism, brood value, prairie and body mass, interactions were significant, and post-hoc tests found that increasing brood value predicted decreasing clutch abandonment in parasitized, large (i.e. low host sensitivity) prairie species (*p*MCMC = 0.028; Figure 2.10). By contrast, brood value had no effect in any other combination of species/nests. No interaction effects were found when considering the forest habitat variable instead of the prairie variable, or when considering incubation time instead of body mass.

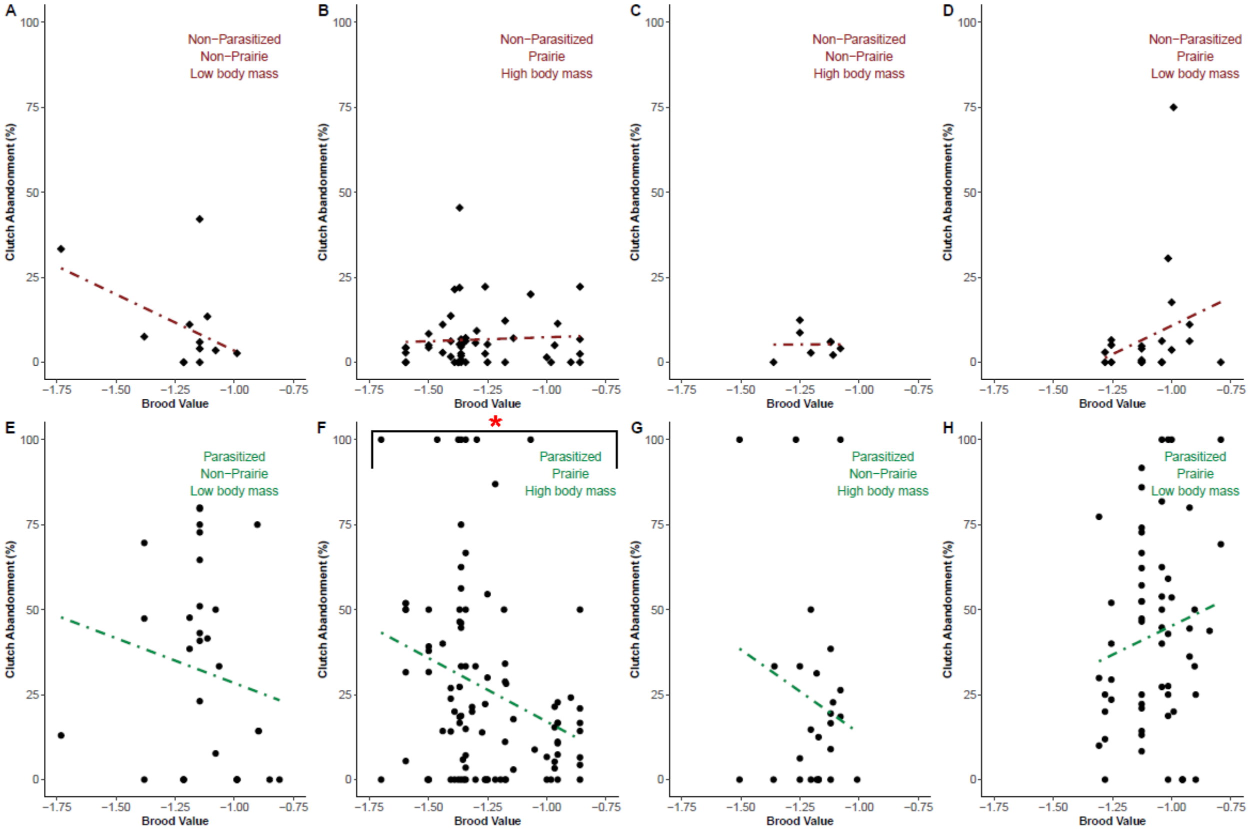

Figure 2.10. Clutch abandonment plotted against brood value for the combined effects of parasitized (E-H) vs non-parasitized (A-D), prairie (B, D, F, H) vs non-prairie (A, C, E, H) habitat, and high (B, C, F, G) vs low (A, D, E, H) body mass. The relationship between brood value and clutch abandonment is significant in high body mass prairie species with parasitized nests (panel F). All other trends are not significant.

<u>Migration effect</u>

Parasitism had a stronger effect on clutch abandonment in non-migratory than in migratory species (*p*MCMC = 0.036; Figure 2.11). There was no interacting effect of migratory behaviour with brood value in predicting clutch abandonment (*p*MCMC = 0.787; Figure 2.12). Nonetheless, migratory species had a higher brood value than sedentary ones (*p*MCMC = 0.006).

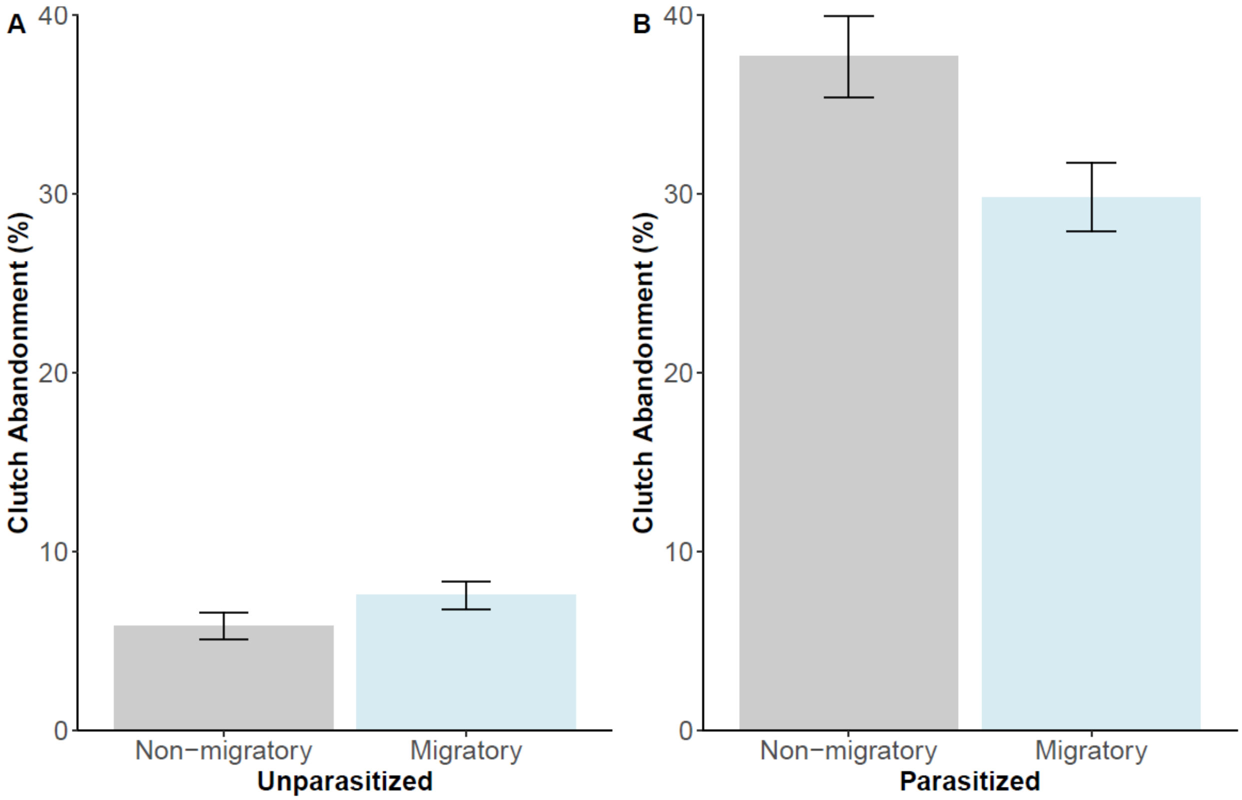

Figure 2.11. Clutch abandonment is shown as a function migratory status for unparasitized (panel A) and parasitized (panel B) populations. Mean + SE. The interaction between parasitism and migration is significant (*p*MCMC = 0.036). Non-migratory species tend to have a lower rejection rate than migratory ones in absence of parasites, but a higher one in presence of parasites. Therefore, parasitism has a stronger effect on clutch abandonment in non-migratory than in migratory species.

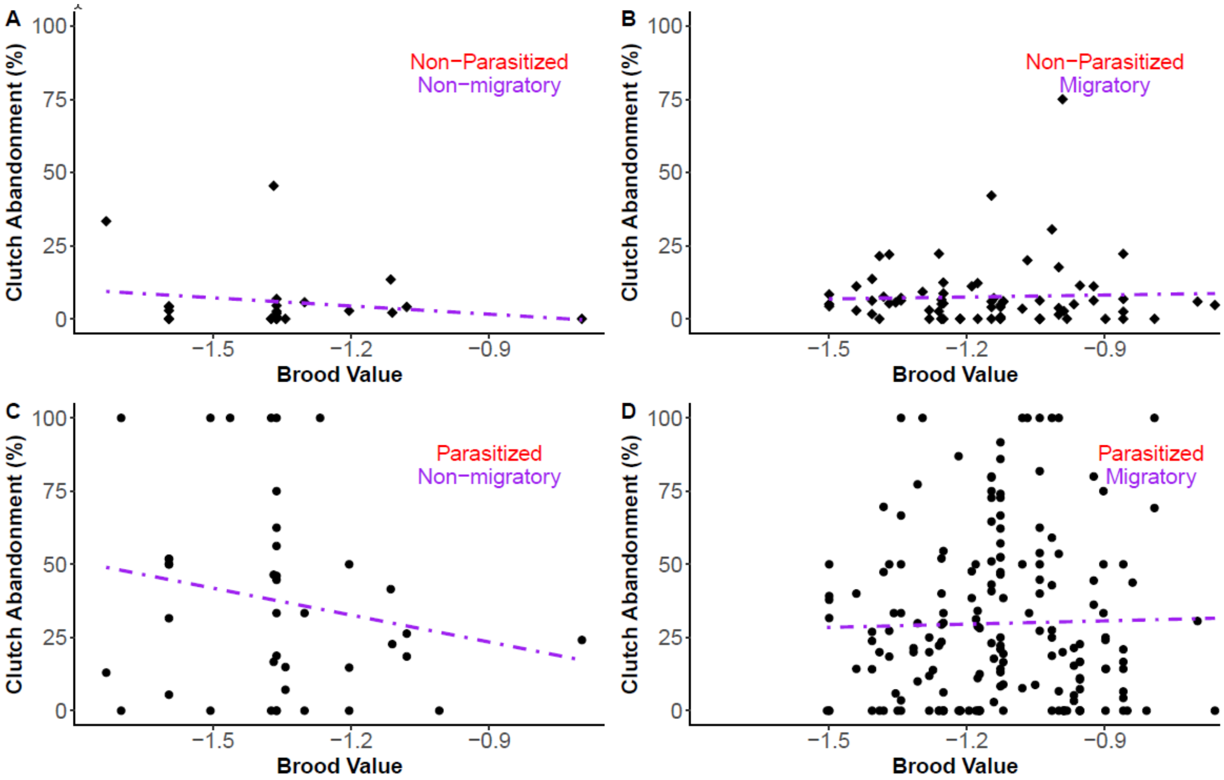

Figure 2.12. Clutch abandonment plotted against brood value for different combinations of parasitized (C and D) vs non-parasitized (A and B) nests and of migratory (B and D) vs non-migratory (A and C) species. No significant relationship between clutch abandonment and brood value is found for migratory or non-migratory species.

Discussion

In this study, we examined life history factors whose interaction may explain the variation in clutch abandonment rate among hosts of the *Molothrus* clade, all obligate brood parasites. Overall, we found that in all cases, parasitism increased clutch abandonment in parasitized populations, and in species with certain life-history traits. The increase was stronger in a habitat with more cowbird-host interaction (open habitat, Figure 2.3). Furthermore, we found that brood value predicted clutch abandonment, but only in specific conditions. In terms of habitat, decreasing brood value predicted increasing abandonment only in unparasitized nests of species breeding in habitats with less cowbird exposure (non-prairie and forest; Figures 2.4 and 2.5, respectively). Species with a lower sensitivity to brood parasitism (i.e. higher body mass) had lower abandonment rates (Figure 2.7). When considering the combined effect of habitat and host sensitivity, we found that when parasitized, high body mass species from a high cowbird-host interaction habitat (prairie) showed increasing abandonment rates with decreasing brood value (Figure 2.10). Migratory status did not contribute to explain variation of clutch abandonment with brood value, but migratory species had a higher brood value than non-migratory species, and clutch abandonment was higher in non-migratory species when parasitized (Figure 2.11).

Small- versus large-scale habitat differences linked with cowbird exposure and response

When considering the effect of parasitism alone on clutch abandonment, we found that while parasitism increases clutch abandonment in all cases, clutch abandonment was lower in forested habitats compared to open habitats, which are habitats with higher cowbird-host interaction (Figure 2.3). Past research has found a similar relationship when comparing parasitism rates between open and forested habitats (Hosoi and Rothstein 2000). This difference has been suggested to be evidence of an evolutionary lag between species that encounter parasitism more often and those whose harder to access breeding habitat shields them to some extent from more frequent parasitism. Our results reinforce these previous findings regarding habitat and parasitism response, and suggest that species with higher parasite-host interactions have more responsive defenses to parasitism.

While brood value alone did not explain clutch abandonment rates found in cowbird hosts on average, brood value explained clutch abandonment depending on parasitism and habitat conditions. Results were consistent between both habitat variables considered, i.e. forest/open and prairie/non-prairie. The prairie/non-prairie variable represents differences in experience with cowbirds at a large scale, both in terms of habitat and in terms of time. Prairie species have historically co-occurred with cowbirds before recent cowbird range expansions and would be expected to be better adapted to respond to parasitism due to their longer evolutionary history. Similarly, the forest/open variable represents a difference in cowbird experience at a smaller habitat scale: species breeding in forest habitats are expected to encounter cowbirds less frequently, and therefore to be less responsive to them, due to both reduced co-evolutionary pressure and individual experience (Soler et al. 2012; Blažek et al. 2018).

For both habitat variables, brood value predicted clutch abandonment, but only in unparasitized nests in species with lower cowbird-host interaction (non-prairie and forest; figures 2.4 and 2.5, respectively). Brood value did not predict clutch abandonment for these non-prairie and forest species when parasitized. By contrast, for host species that evolved with brood parasites (i.e., in open habitats and in prairies), brood value does not predict nest abandonment, regardless of the presence or absence of parasitism.

Because forest species and non-prairie species correspond to categories that are expected to have less experience with brood-parasites and be less adapted to them, our results suggest that species from habitats with higher cowbird exposure having to implement antiparasitic responses may have changed how brood value predicts clutch abandonment. In a similar way, the fact that brood value did not matter when nests were parasitized regardless of habitats suggests that being parasitized alone was enough to affect brood value. In the absence of parasitism, birds may be more likely to abandon their nest if their brood value is low, following life history theory. However, when parasitism comes into play, both at the individual and species level, the effect of brood value is mitigated, and a general increase in the probability of clutch abandonment is observed. These results may suggest that the cost of parasitism is so high that when an individual is parasitized, brood value becomes irrelevant. In short, parasitism nullifies brood value.

Host sensitivity mediates clutch abandonment in specific cases

We expected that some hosts would be more likely to abandon depending on the size of the fitness cost they incur from being parasitized. We examined two variables as proxies for the cost of parasitism: body mass and incubation time. High body mass and short incubation time were both associated with low sensitivity to parasitism, while low body mass and longer incubation time corresponded to high sensitivity. The relationships observed with regards to host sensitivity were specific and statistically significant only in certain groups. Incubation time did not appear to explain clutch abandonment variation (Figure 2.6), which suggests it is not a key element in explaining differences in response to parasitism. Meanwhile, body mass had an effect under certain conditions. Large body mass (i.e. low sensitivity) species were less likely to abandon their clutch in general when compared to small (i.e. high sensitivity) species, regardless of parasitism (Figure 2.7). While this difference was expected in that higher sensitivity species showed increased abandonment rates, the lack of relation to parasitism diverges from our predictions by suggesting that parasitism is not a driver of this difference. The observed greater propensity of small species to abandon in general may suggest that they are more vulnerable to factors impacting breeding in general, such as weather or predation (Robin et al. 2001).

Furthermore, it emerged that, when parasitized, large prairie species exhibited a trend of increasing abandonment with decreasing brood value (Figure 2.10). This relationship was only observed in this particular set of conditions. This negative relationship between brood value and clutch abandonment is consistent with our predictions, however its presence only in large species and in the prairie habitat is not. In line with our observations that brood value did not predict abandonment in species from habitats with higher cowbird exposure, species with a higher host sensitivity may exhibit a comparable effect, whereby the costs of parasitism are so high that the importance of brood value is mitigated, while the effect of brood value would be visible in the group with the least sensitivity to parasitism. Less expected is this relationship being present only in the prairie group, the higher cowbird exposure habitat, as opposed to the non-prairie group. This differs from the habitat-only analyses, where brood value was not important in species more exposed to parasitism. Overall, these results suggest that brood value predicts clutch abandonment in some conditions of lowered host sensitivity where abandonment rates are also generally lower, while depending on complex interactions of habitat and sensitivity factors.

Migratory behaviour is associated with brood value and abandonment

Migratory status did not have an effect on the relationship between brood value and clutch abandonment (Figure 2.12). However, migratory species had a higher brood value and a lower clutch abandonment rate than non-migrants (Figure 2.11). Brood value being higher in migratory species is in line with the expectation that migration puts constraints on breeding (Zink 2011), and validates our assumptions along similar lines in the context of brood parasitism. Although our results correspond to the expected consequences of a negative relationship between clutch abandonment and brood value, this relationship was not supported by our data. There may be additional factors underlying the links between brood value, migration, and clutch abandonment, such as costs of migration not accounted for in our analyses, such as survival or the physical demands of migration (Zink 2011). Overall, our results indicate that migration is linked with increased brood value and decreased clutch abandonment, but these effects are not associated with an effect of brood value on clutch abandonment, suggesting different factors are driving the variation.

Implications for evolutionary lag and equilibrium hypotheses

Our results may be interpreted in different ways in the context of explaining the importance of lifetime reproductive success in determining abandonment rates. Under the evolutionary lag hypothesis, host tolerance of parasitism is explained as being primarily the result of a failure to recognize parasitism. As discussed earlier, the increase of clutch abandonment associated with increased cowbird-host interaction may be supportive of the presence of an evolutionary lag (Hosoi and Rothstein 2000), and parasitism negating brood value may be seen as further evidence that the cost of parasitism precludes any other response than abandonment. However, the lack of effect of brood value in the presence of parasitism may be the result of trade-offs not quantified by brood value mediating the relationship. Breeding individuals also face pressures at the scale of the breeding season rather than the individual's entire lifetime. Notably, the specific timing of clutch onset during a breeding season is a relevant factor, as the cost of clutch abandonment compared to the cost of parasitism acceptance can change over the course of the season (Sealy 1995; Guigueno and Sealy 2010). Indeed, towards the end of a breeding season, conditions rapidly become less favourable for breeding. As a result, the cost of abandonment and delayed re-nesting can grow larger than the fitness cost of accepting parasitism (Guigueno and Sealy 2010). The likelihood of abandonment has also been shown to increase greatly when a cowbird lays its egg before the host lays (Sealy 1995). In the context of this study these short-term timing effects were not included because of the amalgamation of multiple studies, including studies conducted over multiple seasons or lacking clutch onset data. In summary, while our results can provide support for the evolutionary lag hypothesis, they do not disprove the evolutionary equilibrium hypothesis, as the variation in clutch abandonment could be mediated by factors not taken into account by brood value. Further research incorporating clutch timing factors may be valuable in the future.

Additional considerations, conclusions and future directions

Excluding bronzed cowbirds did not change the results, suggesting the interspecific differences were not large enough to indicate different trends among different species. When excluding shiny cowbirds to focus solely on brown-headed cowbirds, there were no effects of brood value on clutch abandonment. This difference may be due to a loss of statistical power, but supported previous results in suggesting brood value has no effect in the presence of parasitism.

Because effects due to clutch onset and timing of parasitism are potential factors, taking them into account in further research on specific hosts' antiparasitic response may be valuable. Migration effects may also potentially warrant their own investigation, to better understand the interactions between brood value, migration and clutch abandonment.

Overall, our results consider a broad dataset to offer several suggestions about how brood value influences clutch abandonment in cowbird hosts in the context of parasitism. Brood value is an important variable to consider to explain differences in clutch abandonment, but brood parasitism has a greater and potentially overriding effect compared to brood value when it comes into play, both at the species and individual level. Species with lower sensitivity to parasitism similarly show an impact of brood value, as complex interaction suggested again that parasitism nullifies brood value in species with a higher host sensitivity. These results offer possible avenues to understand broad life history factors and how they explain variation in host tolerance of brood parasitism, all the while providing insight as to how brood value may inform parental care in a context of lowered fitness.

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Linking statement to Chapter 3:

In Chapter 2, I showed that clutch abandonment is a response to parasitism that is mediated by differences in life history traits between different species. Therefore, Chapter 2 provides evidence that constraints of the environment may be associated with the antiparasitic response they exhibit. While these findings give a broad sense of the factors associated with differences in the response of individuals of a given population compared to other populations, they do not provide insight into how these behaviours are mediated at the level of the individual. For this purpose, in Chapter 3, I take a different but related approach. I focus on a single species, the yellow warbler (*Setophaga petechia*), a common host of the obligate brood parasite brown-headed cowbird (*Molothrus ater*). My aim is to directly investigate the decision to abandon and endocrine and visual factors associated with it.

CHAPTER 3:

CLUTCH ABANDONING PARASITIZED YELLOW WARBLERS HAVE INCREASED CIRCULATING CORTICOSTERONE WITH NO EFFECT OF PAST CORTICOSTERONE OR DIFFERENCES IN EGG MACULATION CHARACTERISTICS

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Abstract

Parental care can be costly to parents' fitness. As such, abandonment of the current reproductive attempt may benefit potential future opportunities, maximizing lifetime reproductive success. Obligate brood parasitism, a reproductive strategy in which parasites lay their eggs in the nests of other species and rely solely on them to raise the parasitic young, is an ideal system to study brood abandonment. Some parasitized host species have evolved anti-parasitic defenses, notably clutch abandonment (egg burial and nest desertion), that may mitigate negative consequences of parasitism. Abandonment of clutches due to parasitism is not unlike abandonment of reproduction in times of stress, suggesting that host responses to parasitism could be triggered at least partly by elevated stress hormones that mediate individual decisions. Yet, the mechanistic basis for clutch abandonment remains unclear. Here, we experimentally parasitized clutches of yellow warblers (*Setophaga petechia*), a common host of the brown-headed cowbird (*Molothrus ater*), with model cowbird eggs to examine whether host circulating corticosterone (CORT) differed among females that accepted parasitic eggs or rejected them through clutch abandonment. We also assessed whether feather CORT, a measure of past corticosterone exposure, differed between acceptors and abandoners. Finally, we investigated whether egg visual signals, specifically differences in maculation characteristics between model cowbird and host eggs, predicted abandonment of experimentally parasitized clutches. Circulating CORT was higher in females who abandoned their parasitized clutches, but not in those who accepted, relative to controls with no egg addition. Past stress and differences in maculation characteristics did not predict whether individuals accepted or abandoned experimentally parasitized clutches. Moreover, differences in maculation characteristics between the host and model cowbird eggs did not predict CORT levels or nest abandonment. Thus, parasitism with subsequent clutch abandonment may be associated with elevated circulating CORT, but neither past stress nor differences in maculation characteristics influenced abandonment. The combination of these results contributes to our understanding of the roles of corticosterone and egg visual signals in the context of clutch abandonment in brood parasitism specifically, and of parental care more broadly.

Introduction

Brood abandonment (Wiggins et al., 1994) occurs in a variety of taxa from insects (Zink, 2003) to birds (Hosoi and Rothstein, 2000). Parental investment theory suggests that a parent should invest in its current brood to the extent that it maximizes lifetime reproductive success, based on a trade-off between current and future reproduction (Klug and Bonsall, 2010; Davies et al., 2012). In some instances, it is beneficial for parents to abandon their current reproductive attempt (i.e., brood abandonment) for the benefit of potential future reproductive opportunities (Davies et al., 2012). In birds, cues that can elicit clutch abandonment vary between and within species and may include predation (Lima 2009; Ackerman et al. 2003), harsh weather conditions (Bottitta et al., 2003; Thierry et al. 2013), and poor body condition (Groscolas et al. 2008; Spée et al. 2010, 2011). Under these challenging conditions, brood abandonment can be adaptive if parents reproduce under more favorable conditions (Sealy 1995; Guigueno and Sealy 2010). Although lifetime reproductive success may be maximized through brood abandonment, abandoners may face extensive costs associated with re-nesting and producing another brood later in the breeding season (Wiggins et al. 1994; Verboven and Tinbergen, 2002; Guigueno and Sealy 2010). As such, brood abandonment represents a key reproductive decision, where abandoners must consider the trade-offs associated with investing in current versus future clutches (Verboven and Tinbergen, 2002; Johnston, 2011; Fokkema et al., 2016; Griesser et al., 2017).

Brood parasitism, in which parasites rely solely on other species to raise their young (Soler 2017; Davies 2000; Feeney et al. 2014), exploits parental care and imposes severe fitness costs on hosts. A parasitized host may incur fitness costs that decrease reproductive success, such as parasitic hatchlings evicting host eggs and nestlings (Soler et al. 1995; Kilner et al. 2004), parasitic hatchlings outcompeting host offspring for food and space (Moskát et al., 2017; Lorenzana and Sealy, 1997) or causing carry-over effects that delay and decrease future nesting attempts in subsequent breeding seasons (Mark and Rubenstein, 2013). To combat these costs, many host species have evolved antiparasitic defenses that reduce the impact of parasitism on their lifetime reproductive success (Rothstein 1990; Davies 2000; Medina and Langmore, 2015). For example, some hosts recognize the specific threat posed by brood parasites and strongly defend the nest against them during the egg-stage when nests are most vulnerable to parasitism, employing front-line defenses (Gill and Sealy, 2004; Lawson et al., 2021). If the parasite successfully lays an egg in the host nest, some hosts may eject it through grasp- or puncture-ejection to avoid raising the parasitic young while not having to abandon a clutch entirely (Servedio and Hauber, 2006). However, the ability to eject parasitic eggs is limited by the ability to visually recognize a parasitic egg (Soler et al., 2017) and morphological characteristics (Rasmussen et al., 2009; Guigueno and Sealy, 2011; Soler et al., 2015). Birds must have a bill of sufficient gape-size and strength to either grasp the parasitic egg or puncture it (Spaw and Rohwer, 1987; Picman 1989). An alternative antiparasitic defense is clutch abandonment.

In clutch abandonment, hosts abandon clutches in response to brood parasitism. The abandoned clutch may consist of only parasitic eggs, or it may include a mix of parasitic eggs and their own (Hosoi and Rothstein, 2000; Soler et al., 2015). Hosts abandon their clutches via either nest desertion followed by renesting at a new site, or burial of the parasitized clutch followed by a new clutch laid in a superimposed nest (Guigueno and Sealy, 2010). Abandonment is costlier than egg ejection in terms of energy and time invested, as host eggs may be lost and nests need to be rebuilt (Clark and Robertson, 1981; Sealy 1995; Moskát and Honza, 2002; Guigueno and Sealy, 2009; Soler et al., 2015). Clutch abandonment varies among species, as well as within and between individuals of the same species (Hosoi and Rothstein, 2000; Guigueno and Sealy, 2010). Only some host species reject parasitized clutches by abandonment, and within these species, individuals may vary in the forms of egg rejection they employ, using for example, ejection or desertion (Servedio and Hauber 2006; Honza et al., 2007) or nest desertion and egg burial (Sealy 1995). The underlying mechanisms that explain within- and between-species variability in responses and the cues that trigger parents to abandon their nests remain unexplained (Abolins-Abols and Hauber, 2018; Avilés, 2018).

Hormones play a crucial role in modulating behaviour (Tata, 2005) and mediate many behavioural adjustments in birds, including antiparasitic responses (Bonier et al., 2009 Ruiz-Raya et al., 2018; Abolins-Abols and Hauber, 2020). Specifically, the glucocorticoid hormone, corticosterone (CORT), is a principal mediator of the behaviours of birds as it increases in response to environmental stressors (Siegel 1980). CORT is often used as an index of stress, although it is only one part of the integrated stress response and is an approximation of whole animal “stress” (MacDougall-Shackleton et al., 2019). Nevertheless, elevated CORT is associated with behavioural changes that promote individual survival (Wingfield 2003; Angelier et al., 2009; Crossin et al., 2012), such as increasing vigilance while decreasing expression of reproduction-related behaviours (Wingfield et al. 1998; Wingfield 2003; Schoenle et al. 2017). Therefore, CORT represents a promising candidate to explore as a proximate mediator of host responses to brood parasitism (Bókony et al. 2009). Simply the presence of a parasitic egg in the nest may increase the host's circulating CORT levels (Mark and Rubenstein 2013; Schoenle et al. 2017; Ibáñez-Álamo et al. 2012) and recent evidence suggests that antiparasitic defenses may be partly mediated by CORT. Abolins-Abols and Hauber (2020) experimentally manipulated CORT levels in egg grasp-ejecting American robins (*Turdus migratorius*) and found that parasitized clutches were more likely to be accepted when potential increases in baseline CORT levels were inhibited. As baseline CORT changes in response to experimental parasitism, it thus can provide further insight into the mechanisms mediating a host's response, including clutch abandonment (Ruiz-Raya et al. 2018; Landys et al. 2006; Scharf et al., 2021).

Individual allostatic load (one interpretation of "stress") is related to CORT levels at both short- and long-time scales, with acute (relatively short-term) responses reflected in circulating levels. It is possible that stressful events earlier in the life of an individual influence current behaviours as well. Analyses of CORT in feathers provide a long-term measure of CORT inferring past stress during feather growth over the moulting period (Bortolotti et al., 2008; Bortolotti et al., 2009; Sherriff et al., 2011). During feather growth when cell differentiation is occurring, circulating CORT continually diffuses in a time-dependent manner through the blood quill into highly vascularized follicles along the entirety of the feather (days-to-weeks, Lattin et al., 2011; Jenni-Eiermann et al., 2015; Aharon-Rotmann et al. 2021; Freeman and Newman 2018). Feather CORT can thus be used as a biomarker for an integrated measure of CORT secretion representing stress levels over a longer period, rather than to a specific environmental stressor as revealed by measures of circulating CORT from plasma (Fairhurst et al., 2013). Hence, feather CORT, representing general conditions from a different phase of the annual cycle, may be associated with how individuals respond to current reproductive decisions due to cumulative allostatic load over time (Martinez-Padilla et al., 2013; Hansen et al., 2016). This idea is analogous to the developmental stress hypothesis, which predicts that stress early in life can affect behaviour and stress responses later in life, such as a major reproductive decision like abandoning a clutch (Nowicki et al., 2002; Spencer et al., 2011).

For a CORT response to be initiated, a stressor must appear that causes the hypothalamus to initiate the hypothalamic-pituitary-adrenal cascade leading to CORT secretion. For brood parasite hosts, one such stressor may be the visual recognition of a host egg, as visual differences in colour or maculation between host and parasite eggs often drive host responses (Rothstein, 1974; Rothstein, 1990; Brooke and Davies, 1988; Lyon, 2003; Manna et al., 2017; Gómez, et al., 2021). The ability of a parent to successfully recognize their own eggs or a discordant egg within their clutch and reject a parasitic egg or parasitized clutch should be adaptive, as the host would no longer incur the costs associated with brood parasitism (Hauber et al., 2019; Ducay et al., 2021). However, visual signal recognition ability may largely rely on the extent of differences in maculation between parasitic and host eggs (Rothstein, 1982; Lahti 2006; Hanley et al., 2019; Honza and Cherry, 2017; Cassey et al. 2008; Spottiswoode and Stevens 2010). Cuckoo egg rejection increases with differences in appearance, such as extent of maculation and spot size, between cuckoo and host eggs (Davies, 2000). Appearance-based discrimination also occurs in cowbird hosts (Segura et al., 2016; Dainson et al., 2017). Thus, the ability to recognize a parasitic egg may initiate rejection via egg ejection, but the role of differences in egg visual signals in the context of clutch abandonment is less clear (Davies and Brooke, 1989a; Langmore et al., 2005; Spottiswoode and Stevens 2010). In addition to egg visual signals stimulating rejection, they may also affect circulating CORT levels. Indeed, Eurasian blackbirds have higher circulating CORT levels when parasitized with non-mimetic eggs (Ruiz-Raya et al., 2018). Hence, clutch abandonment may then be mediated by increases in circulating CORT initiated by the extent of maculation characteristic differences between parasitic eggs and their own.

The yellow warbler (*Setophaga petechia*, hereafter “warbler”) is a common North American passerine that is frequently parasitized by the brown-headed cowbird (*Molothrus ater*, cowbird hereafter). They are the only host of the cowbird known to regularly reject parasitized clutches by two forms of clutch abandonment: nest desertion and egg burial (Clark and Robertson, 1981; Sealy, 1995, Mico 1998; Hauber et al. 2006; Guigueno and Sealy 2012). About 35% of female yellow warblers bury cowbird eggs, sometimes with their own eggs, and attempt to re-nest, whereas 12% of females desert the nest entirely and renest at a new site (Sealy 1995). If parasitism occurs early in the laying period, roughly 50% of females bury (Clark and Robertson 1981). However, warblers rarely desert when parasitized during incubation, possibly due to significant costs related to time lost and building an entirely new nest (Guigueno and Sealy, 2009). In addition, warblers are more likely to bury or desert parasitized clutches early in the breeding season when there is time to re-nest (Guigueno and Sealy 2010). Due to high costs of clutch abandonment, warblers may rely on multiple stimuli when making their decision, such as interaction with the adult parasite at the nest (Guigueno and Sealy, 2011). Whereas the colours of the egg background (off-white) and maculation (dark brown) are similar between warbler and cowbird eggs (Guigueno and Sealy, 2009; Guigueno et al. 2014), the extent of differences in maculation characteristics (maculation cover and spot profile) between cowbird and warbler eggs have not been quantitatively measured despite obvious qualitative differences noted in the field.

Here, we experimentally parasitized nests of warblers with model cowbird eggs to investigate the relationship between CORT levels (current circulating via plasma and past via feathers), differences in maculation characteristics, and abandonment of parasitized clutches. As circulating CORT can change rapidly in response to environmental stressors (Wingfield 2003), we predicted that circulating CORT would be higher in females who accepted (1a) model cowbird eggs, and (1b) those who abandoned experimentally parasitized clutches compared to controls whose clutches are monitored without being experimentally parasitized. Given that acceptors and clutch abandoners both received model cowbird eggs, we expected that individuals who experienced elevated past CORT would have an amplified current stress in response to the presence of parasitic eggs (Martinez-Padilla et al., 2013; Hansen et al., 2016). Control individuals and individuals who received an egg each come from a random sample, but within the group of individuals who received an egg, abandoners and acceptors would presumably split non-randomly if past stress played a role. Therefore, (2a) we reasoned that control individuals would have intermediate feather CORT levels between accepters and clutch abandoners with a greater variance than each of the two experimentally parasitized groups. We also explored two alternatives that (2b) individuals that abandoned would have increased feather CORT levels, as past stress would interact with present stress and increase the probability of clutch abandonment and that (2c), individuals that accepted would have increased feather CORT compared to abandoners, because if an individual experienced long-term stress and carry-over effects existed, hosts would not be able to pay the costs of abandonment and thus, individuals with higher feather CORT would be more likely to accept. As such, we predicted significant differences in means and variance among all three groups (control, acceptors, abandoners), with the greatest difference between abandoners and accepters. Finally, because differences in egg visual signals between cowbird and host eggs could potentially influence circulating CORT and the probability of abandoning a parasitized clutch (Ruiz-Raya et al., 2018), we assessed the extent of differences in egg maculation characteristics between warbler and model cowbird eggs added to clutches. We predicted that (3a) circulating CORT would be positively associated with the extent of differences in egg maculation characteristics between host and model cowbird eggs and (3b) acceptors would have smaller differences in maculation characteristics compared to clutch abandoners. The combination of these analyses provides us with a fuller understanding of the effects of hormones and egg visual signals in the context of clutch abandonment in brood parasitism specifically, and of parental care more broadly.

Methods and methods

Study design

The study was conducted within a radius of 35 km of Kalamazoo, Michigan, United States (42°17′24″N, 85°35′09″W) between May-June in 2010, 2011, and 2019. Nest sites were situated in habitats consisting of marshes and shrubs where yellow warblers were abundant, and cowbirds were observed daily.

We found nests by observing singing males and females carrying nest-building materials. We sought to find nests as early in the breeding season as possible, ideally in the preliminary nest building stages, to ensure proper timing of experimental parasitism. We visited nests during the building stage every two days until nest-lining was complete (Figure 3.1a). We then monitored the nests daily to identify the day of the first egg laid (Figure 3.1b). Eggs were numbered in their laying order with a non-toxic permanent marker. Because natural parasitism by cowbirds is too infrequent at our field sites to provide a sufficient sample size, we made puncturable artificial eggs out of shaping floral foam covered in a thin layer of plaster of Paris, closely mimicking cowbird eggs in shape, colouration, weight and volume (see Guigueno et al., 2014). The eggs were painted off-white with brown maculation using waterproof acrylic paints, so that the surface presents a cloud of dark brown spots spread across a dirty white surface (Guigueno et al. 2014; Figure 3.1 c-f). Two model cowbird eggs were added to each experimental nest, which does occur naturally (Kuehn, 2009), to increase the likelihood that females detected the clutch manipulation. On laying day one (Figure 3.1b), we added two cowbird eggs to the warbler's clutch (Figure 3.1c) between 6 am and 12 pm (Eastern Daylight Time) to correspond approximately to the female cowbirds' egg laying window. The nests were monitored daily over the next six days to observe laying progression and to record acceptance (Figure 3.1d-f) or clutch abandonment via nest desertion or burial. Egg burial was recognized by the slight pushing down of the eggs and/or the addition of lining material over the eggs, while desertion was recognized when the female was not observed at the nest for three days in a row and eggs were cold (Guigueno and Sealy, 2010). One out of every three nests found were randomly chosen to act as controls and did not receive model cowbird eggs. Control nests were monitored at the same frequency as the experimental group. If a nest was naturally parasitized, the nest was excluded from the study, to keep a consistent protocol.

Figure 3.1 (a-f). The stages of the experiment conducted on yellow warbler nests near Kalamazoo, Michigan, from initial completion of building of the nest to laying day four. A) a completed nest in the final building stage; B) Laying day one, when we artificially parasitized the clutch; C) A nest after we added two model cowbird eggs to the clutch on laying day one; D) Laying day two; E) Laying day three; F) Laying day four, days of host blood and feather sampling.

Circulating plasma corticosterone

Female warblers abandon on average three days after parasitism, with burials occurring after 2.3 ± 0.1 days and nest desertion after 2.5 ± 0.3 days (mean ± SE, Guigueno and Sealy 2010). Therefore, we collected a blood sample on laying day four to capture a snapshot of the female's circulating CORT during this critical period. We captured females between 8:00 and 14:00 (Eastern Daylight Time). This time window was chosen to minimize time of day differences among individuals, while giving time to capture hard-to-capture birds and to sample multiple females on the same day. We sampled a small number of females after 12:00 pm to ensure that we collected blood samples on the appropriate day in the female's laying cycle. On laying day four (Figure 3.1f), mist nets were placed near the focal host nest to passively capture the female. We then aimed to collect a blood sample from the alar vein within three minutes of capture, recording the time from capture to sample acquirement. In nine cases, sampling time exceeded three minutes, which would increase CORT due to acute stress of capture (Romero and Reed 2005); therefore, we ran a correlation analysis between capture time and CORT level of all individuals and estimated at three minutes the CORT level for those females whose sampling time was prolonged. Each female was banded with a numbered United States Fish and Wildlife Service aluminum band and a unique combination of colour bands before being released to ensure we did not resample females within or between years. Blood samples were kept on ice until they were centrifuged to separate plasma from red blood cells. The separated plasma was pipetted into a separate labelled vial and frozen in a -20 Celsius freezer for long-term storage.

Plasma corticosterone was determined using an enzyme-linked immunosorbent assay (ELISA) kit from ENZO Life Sciences, Inc. (Farmingdale, New York), following kit instructions. This ELISA has been previously validated and used to measure plasma corticosterone in multiple songbirds including sparrows (Wada et al, 2007; Ouyang et al, 2021), wrens (Strange et al, 2016), finches (Kraft et al, 2021) and swallows (Sarpong et al, 2019). Plasma samples were diluted to fall within the range of the standard curve, generally 1:40 or 1:50, incubated with 1% steroid displacement reagent, and run in triplicate or duplicate depending on the sample volume. All samples from a single year were run together on the same plate during the year of sample collection; the intra- and inter-assay coefficients of variation were less than 10%. Assay sensitivity was calculated to be 20.34 pg/ml and all samples read above that value.

Feather corticosterone

During capture of the female warbler on the fourth laying day (Figure 3.1f), the outermost rectrices (R6) on both sides of the tail were collected from the female warbler. We included both second-year and after second-year individuals within our study. Second-year individuals grew feathers during their juvenile moult in the nest, while after second-year birds grew rectrices feathers as part of their complete moult on the breeding grounds between June and September (Pyle 1997). As such, birds within both age-classes grew sampled feathers within the previous breeding season, but, in non-overlapping timeframes. As such, we tested for differences between second-year and after second-year individuals using a linear model on R Statistical Package (R Core Team 2020), with age as a predictor and feather CORT as the dependent variable. As there were no significant differences between age and feather CORT ($p = 0.61$), we merged the two groups for subsequent analyses.

Extraction of corticosterone from feathers was conducted following a procedure similar to that previously described in Bortolotti et al. (2008). The calamus was removed and the length and weight of the feather were recorded using a digital caliper and an analytical balance, respectively. The feather (vane and rachis) was cut and minced into small pieces and put into a 50 ml conical tube with 10 ml of methanol. The tubes were sonicated for 30 minutes in a sonicating bath and then incubated overnight at 50°C in a shaking water bath. The next morning, the methanol was filtered through a 70 µm cell strainer and further filtered through a 0.22 µm cellulose acetate syringe filter into a glass tube. Methanol was evaporated by placing tubes into a 50°C shaking water bath in a fume hood. Once evaporated, samples were reconstituted with 1 ml ELISA assay buffer with gentle rocking for 3 hours. Samples were transferred to microcentrifuge tubes and frozen at -20°C until assayed no more than 7 days later.

Feather corticosterone was determined using an ELISA from ENZO Life-Sciences Inc, following the kit instructions with the extension of the standard curve by one value on the low end (total range: 20,000 – 6.4 pg/ml). All samples from a single year/season were run on the same plate in triplicate without dilution. Assay sensitivity was 6.4 pg/ml and all samples read well above this value. Intra- and inter-assay coefficients of variation were less than 10%. Corticosterone values were normalized to feather length.

Egg visual signals

On laying day four (Figure 3.1f), we collected the last laid egg. Some nests were inaccessible because of height or heavy vegetation and as a result, it was only possible to determine clutch size but not possible to either mark or sample the eggs, resulting in smaller sample sizes for eggs when compared to circulating CORT samples collected from females (Table 3.1).

Table 3.1. Summary of CORT and egg visual signals measurements organized by treatment. "Accepted" are experimentally parasitized clutches accepted by the warbler, "Abandoned" are those that were either buried or deserted, and "Control" are the nests monitored without being experimentally parasitized.

Factor	Egg added		Control	Total sample size
	Accepted	**Clutch abandoned (burial or desertion)**		
Plasma CORT	19	6	15	40
Feather CORT	16	5	8	29
Egg visual signals	12	3	NA	15

We followed a similar egg image analysis procedure as Hauber et al. (2018). The fourth laid warbler eggs were each compared to one standardized model cowbird egg. Eggs were placed on their side on a colour standard card with a ruler and were photographed with a digital camera mounted on a tripod. The eggs were each photographed four times, with a 90-degree rotation along the axis between photos. Image analysis on warbler and model cowbird eggs was done using Adobe Photoshop®. We first cropped each egg image into three sections along its long axis: (1) apex-end third, (2) middle-region third, and (3) blunt-end third. We quantified maculation coverage percentage within each third by converting the maculated area to black (RGB = 0,0,0) and the non-maculated area to white (RGB = 255,255,255). We then measured the percentage of black pixels within each third. We obtained the maculation coverage percentage for each third by finding the mean of the percentages between the four images for each egg. We calculated the absolute difference in maculation coverage percentage between the blunt and apex ends for all eggs, thus producing a measure of end-to-end maculation coverage variation. The model cowbird egg's end-to-end maculation coverage value was then subtracted from the warbler egg's maculation coverage, providing a measure of the end-to-end difference in maculation coverage between these two eggs. As such, a positive value signified that the host egg had greater variation than the cowbird model egg and a negative value would indicate that there was less variation.

We obtained an average spot length-to-width ratio of the apex and blunt thirds by randomly selecting five spots within each third of each egg image. The mean spot ratio within each of the apex and blunt thirds of the egg was then averaged across the four images for each egg, which we termed average spot profile. We then calculated the absolute difference in spot profile between the blunt and apex ends for all eggs, to have a consistent measure of within-egg end-to-end variation. We used the absolute spot profile difference for both the cowbird model egg and host egg. Finally, we subtracted the model cowbird egg's absolute difference in within-egg spot profile variation from that of the host egg, producing either a positive or negative value. As such, positive value signified that the host egg had greater spot profile variation than the cowbird model egg and negative values indicated there was less variation. These measures of end-to-end maculation and spot profile variation were chosen for our analysis because we expected them to be most representative of the difference between yellow warbler and cowbird eggs, due to the gradient in maculation density observed on yellow warbler eggs but not on cowbird eggs (Guigueno et al. 2014).

Statistical analysis

All statistical analyses were conducted on R Studio (2021) with R Core Team (2020). Data were categorized into three treatment groups; controls, accepters, and abandoners, as detailed above. All graphs were constructed using '*ggplot2*' (Wickham, 2016) and '*ggsignif*' (Ahlmann-Eltze, 2021) packages for R. All data are presented as mean ± SE.

Circulating CORT data were normalized using a log-transformation. There was one unusually small outlier that we corrected to one, which was still the lowest data point, to avoid deformations in the log-transformation. We had strong *a priori* directional predictions for plasma corticosterone variation (1a) and (1b), expecting higher CORT levels in experimental treatments compared to controls. As such, we used one-tailed Student's t-test (using the *'t.test'* function) to detect differences between groups. To test the prediction that egg addition alone would result in higher CORT in acceptors than in controls (1a), we excluded the effect of clutch abandonment. To test the prediction that circulating CORT was associated with clutch abandonment (1b), abandoners were compared to controls. Because variance was similar between accepters and controls, but about half for abandoners versus controls, we ran t-tests with homogenous versus heterogeneous variances for predictions (1a) and (1b), respectively.

Length-normalized feather CORT data was subsequently log-transformed to meet the assumption of normality. Levene's test was used to test for homogeneity of variance between the treatment groups using *'leveneTest'* function of the *'car'* package (Fox and Weisberg, 2019). To investigate the relationship between accepted, abandoned and controls clutches we used a generalized linear model using the '*glm*' function. If significant, we did post-hoc tests between all three groups to test predictions 2a and 2b that increased feather CORT would be associated with either increased abandonment or increased acceptance.

For egg visual signals, analyses were conducted using only eggs from experimentally parasitized nests, where the warblers would have been confronted with the sight of the model cowbird eggs. We ran two linear models using the 'lm' function. The dependent variable for both models was log-CORT, and the predictor variable was the difference in either within-egg maculation coverage variation or within-egg spot profile variation (expressed as the absolute difference between the model and host eggs). Additionally, we used one-tailed Student's two-sample t-tests ('*t.test'* function in R) to determine whether maculation coverage and spot profile variation were greater among warblers that abandoned their experimentally parasitized clutch relative to those who accepted.

Results

Our sample consisted of a total of 40 yellow warbler nests, with 25 experimentally parasitized and 15 control nests (Table 3.1). Within the experimentally parasitized group, 19 females accepted the experimental parasitism (76%) and 6 abandoned their parasitized clutch (24%) through either desertion (*n* = 4) or burial (*n* = 2).

Circulating plasma corticosterone

A total of 40 circulating CORT samples were collected (Table 3.1). Circulating CORT levels of accepters were not different than controls ($t = 0.45$, df = 33, $p = 0.33$; Figure 3.2a). In contrast, circulating CORT levels of abandoners were significantly higher than controls ($t = 1.96$, df = 15, $p = 0.03$; Figure 3.2b).

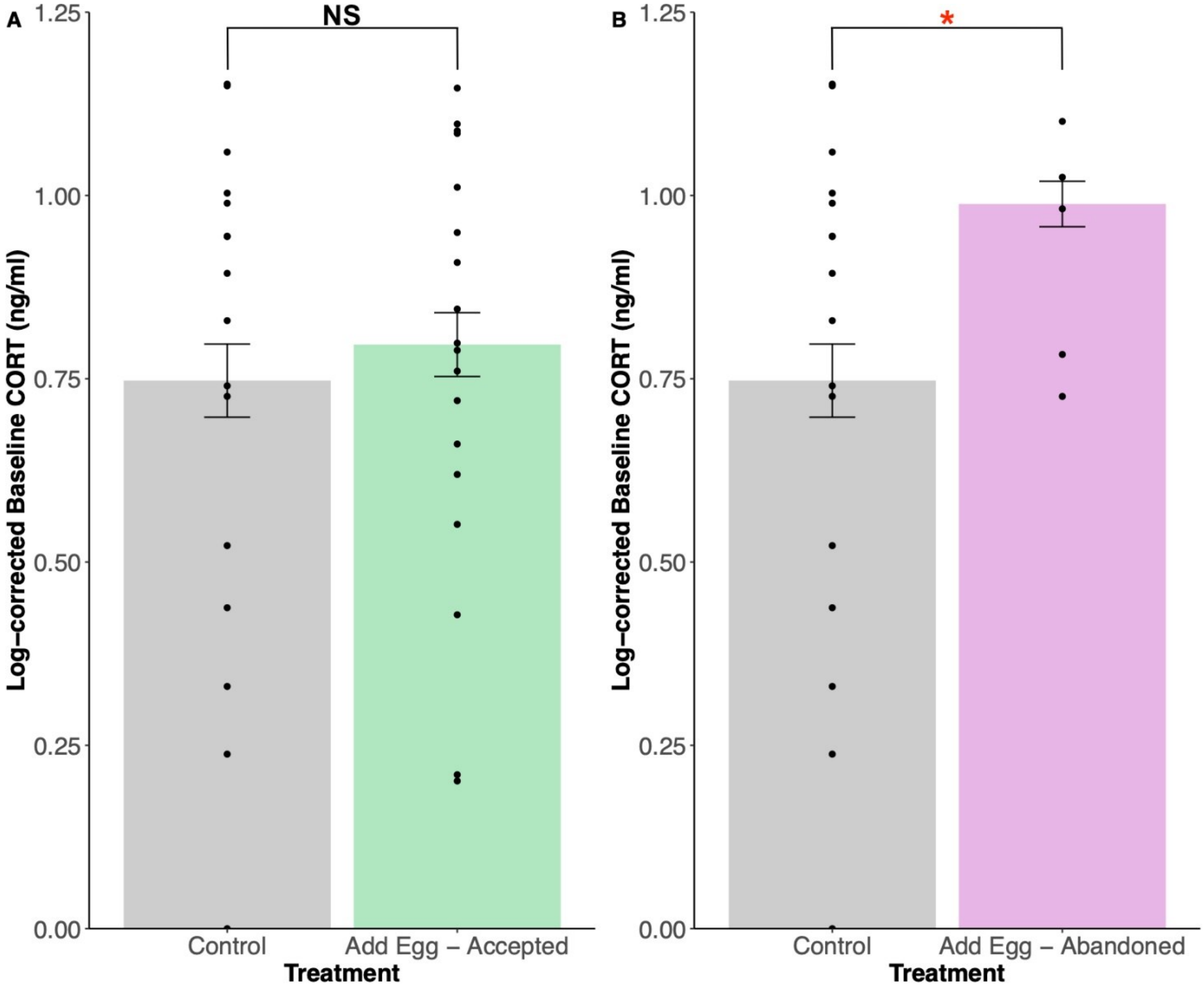

Figure 3.2 (a-b). Comparisons in log-corrected, circulating corticosterone (CORT) levels in circulating plasma of controls (no model eggs added to clutches) and two experimental treatments (model eggs added). **a)** Individuals who accepted the cowbird model eggs did not have significantly higher baseline CORT than controls. **b)** In contrast, individuals who

abandoned their experimentally parasitized clutches has higher circulating CORT than controls. NS and * indicate “not significant” and $p < 0.05$, respectively. Mean ± SE.

<u>Feather corticosterone</u>

Feather samples from 29 individuals were collected (Table 3.1). Variances were similar across treatments ($F_{2,26} = 0.43$, $p = 0.65$; Figure 3.3). Mean feather CORT concentrations were similar among treatment groups ($F_{2,26} = 0.11$, $p = 0.89$; Figure 3.3); as such, post-hoc tests were not conducted.

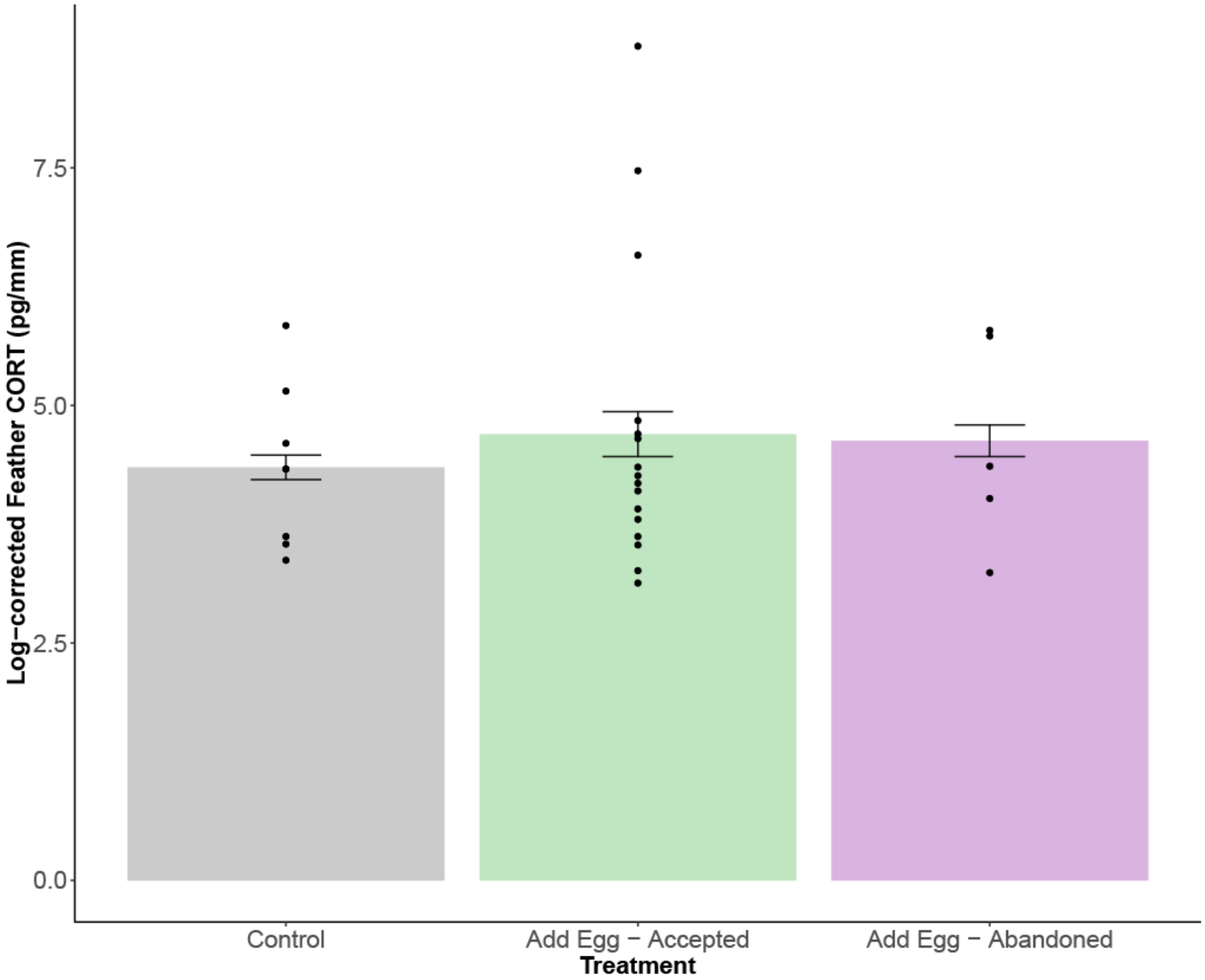

Figure 3.3. Log-corrected feather corticosterone (CORT) levels (pg/ml) in feathers of females across the three experimental groups. There was no significant difference in the variance or the mean between groups. Mean + SE.

Egg visual signals

In total, 22 warbler eggs were analyzed (Table 3.1). The model cowbird egg had an end-to-end maculation coverage variation of 6.6% and an end-to-end spot profile variation ratio of 0.04. Contrastingly, the warbler eggs had an average end-to-end maculation coverage variation of 33.3 ± 2.32% and an average end-to-end spot pro file variation ratio of 0.16 ± 0.07.

Circulating CORT levels were not associated with differences in within-egg maculation coverage variation between cowbird model and host eggs ($R^2 = -0.05$, $p = 0.44$; Figure 3.4a), nor were they associated with spot profile ($R^2 = 0.09$, $p = 0.27$; Figure 3.4b). Including treatment (accepted versus abandoned) as an explanatory variable did not change the results. Further, differences in maculation coverage (df = 13, $t = -0.43$, $p = 0.66$) and spot profile (df = 13, $t = -0.01$, $p = 0.50$) variation between eggs were not greater in abandoners compared to accepters (Figure 3.5a-b).

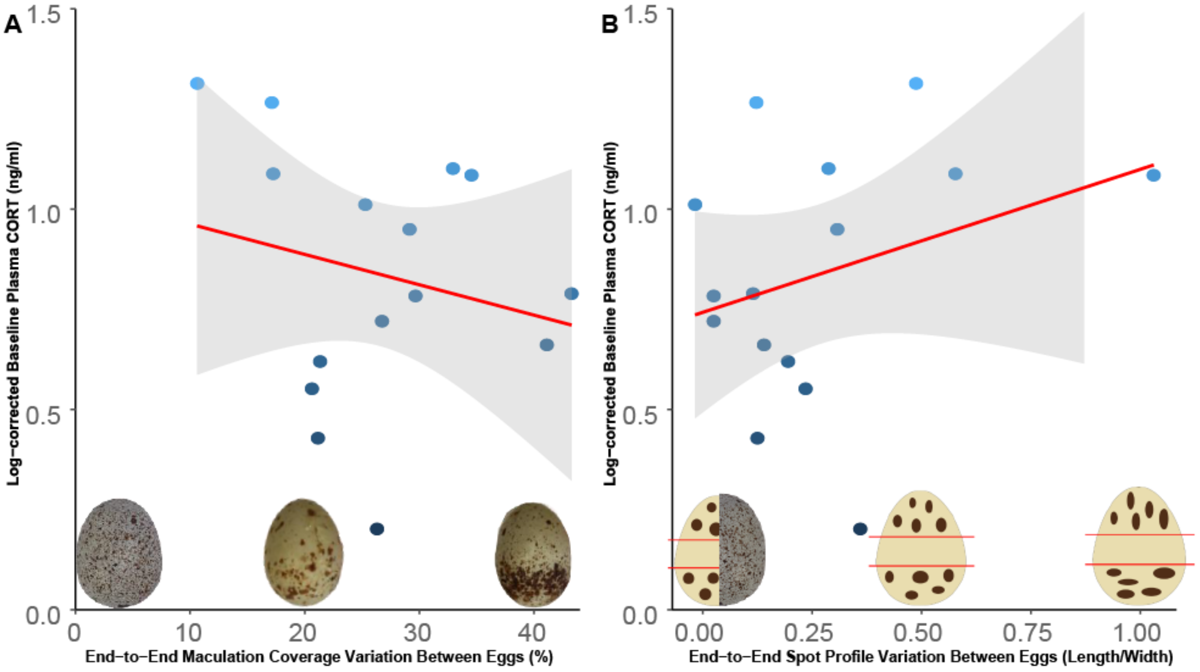

Figure 3.4 (a-b). Correlation between log-corrected circulating CORT levels from individual female warblers increasing variation in **a)** maculation coverage and **b)** spot profile of their eggs relative to the model cowbird egg**.** The shaded area indicates 95% confidence interval of the mean slope.

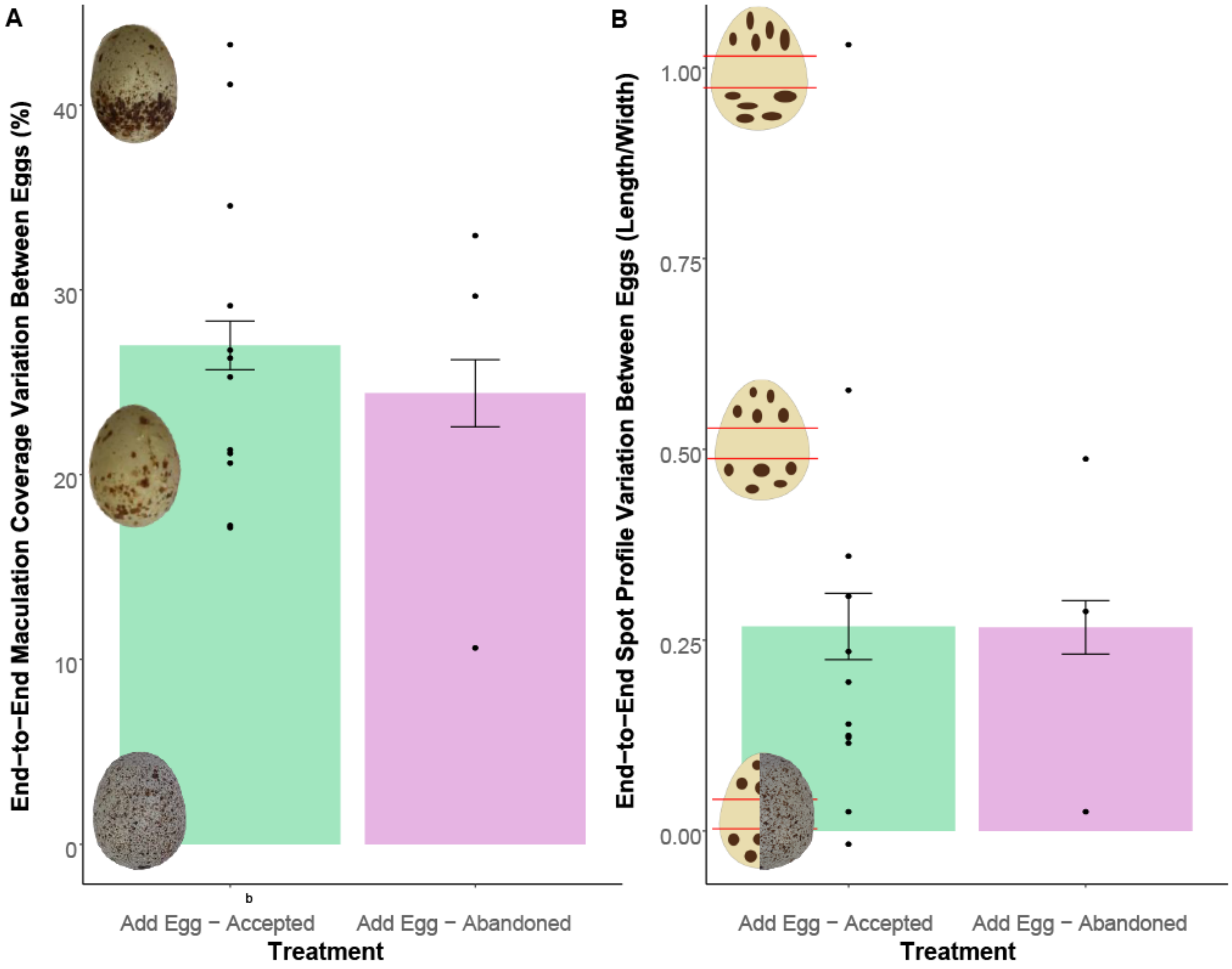

Figure 3.5 (a-b). End-to-end egg visual signal variation by treatment. Females who abandoned their experimentally parasitized clutch did not have eggs that varied more in **a)** maculation coverage or **b)** spot profile relative to the model cowbird egg. Mean + SE.

Discussion

In this study, we examined mechanisms associated with clutch abandonment in a frequently parasitized host of the brown-headed cowbird, the yellow warbler, which regularly rejects naturally parasitized clutches via egg burial and nest desertion (Sealy 1995). Circulating CORT levels were higher in females who abandoned their parasitized clutches, either by egg burial or nest desertion, relative to females at control nests in which no model cowbird eggs were added (Figure 3.2b). Simply adding model cowbird eggs to clutches did not increase circulating

CORT, as females that accepted cowbird eggs did not have elevated CORT relative to controls (Figure 3.2a). In addition, past, long-term stress, as approximated by feather CORT, did not predict whether females accepted or abandoned experimentally parasitized clutches (Figure 3.3). Finally, differences in egg visual signals, as measured by differences in maculation coverage and spot profile variation between the model cowbird egg and host eggs, did not predict abandonment nor were they correlated with circulating CORT levels of females who abandoned their nests (Figures 3.4, 3.5).

Several explanations for the increased CORT in abandoners relative to controls are possible. First, females with naturally higher circulating CORT may be more likely to abandon their nests, such that the presence of a parasitic egg might not alter host CORT levels. That is, individuals with high CORT are more predisposed to abandon for any reason, and the presence of a parasitic egg may tip them past the threshold of abandonment. Second, the presence of a parasitic egg might increase CORT, but only in some females, which then abandon the nest. This alternative is consistent with our findings that circulating CORT levels in females that accepted experimental parasitism did not differ from control females, whereas abandoners showed higher CORT. Third, a parasitic egg might increase CORT in all females, but females whose CORT levels exceed a certain threshold are more likely to abandon the nest than those whose CORT remains below the threshold. We do not believe that this final explanation is likely because there was no difference in CORT between control birds and accepters. Fully resolving these differences would require blood samples before and after parasitism, preferably coupled with an experimental increase in CORT (i.e. implants). These approaches would require capturing females multiple times during laying, which may substantially increase abandonment, thereby overwhelming any experimental effect due to experimental parasitism.

Circulating corticosterone (plasma)

Circulating glucocorticoids, such as CORT, are plastic and fluctuate in response to environmental stressors (Siegel 1980; Wingfield 2003). In our study, females who abandoned their parasitized clutch had higher circulating CORT, relative to females with control clutches that were not parasitized (Figure 3.2b). Moreover, circulating CORT did not differ between control and acceptor females. Females were sampled three days (laying day 4; Figure 3.1f) after the clutch manipulation (laying day 1; Figure 3.1c), suggesting that abandoners in the population were associated with a more pronounced endocrine response than accepters (Figure 3.2a). Given that clutch abandonment is a generalized response, similar increases in circulating CORT could be found in association with other abandonment-triggering factors, such as inclement weather (Romero et al., 2000, Thierry et al, 2013). Therefore, circulating CORT could provide us with a broader understanding of how environmental stressors, whether biotic or abiotic, affect nest abandonment in birds.

Brood parasitism *per se* does not appear to affect circulating CORT levels in female yellow warblers. Our results show that simply adding model cowbird eggs, without the host abandoning the clutch, was not associated with higher circulating CORT relative to control females. The absence of a difference between controls and accepters, contrary to our prediction, is intriguing, as it suggests that females who did not abandon experienced a comparable level of stress compared to unparasitized (control) individuals, a finding that differs from previous research. For instance, a common cuckoo host, the Eurasian blackbird, experienced increased circulating CORT following experimental parasitism (Ruiz-Raya et al. 2018). The difference in findings could depend on several factors including parasitism rates, predation rates, breeding season length, and other life history traits (Bókony et al., 2009). The fact our study focused on a species with a variable abandonment response to parasitism may explain the different pattern of circulating CORT variation that we observed compared to species with more consistent responses such as Eurasian blackbirds (Ruiz-Raya, et al. 2018). Our results are in line with the findings of Abolins-Abols and Hauber (2020), who found a causal link between stress-induced CORT and egg ejection rate in American robins, an egg-ejector species. Together, these findings suggest circulating CORT may mediate antiparasitic responses across different parasite-host systems and across rejection methods (Mark and Rubenstein 2013; Schoenle et al. 2017; Ibáñez-Álamo et al. 2012).

Past corticosterone (feathers)

Feather CORT, in contrast to circulating plasma CORT, indicates a long-term past “stress” history (Bortolotti et al. 2008), integrating levels of circulating CORT across the moult period. Our predictions (2a and 2b) that abandoners and accepters would have higher and lower past CORT than controls, respectively, focused on whether an individual’s prior state could predict a response to the future stressor of experimental parasitism. As such, these predictions resemble those of the developmental stress hypothesis, which proposes that stress early in life (e.g., nestling nutritional state) can affect behaviour and cognition later in the life (e.g., adult song quality). Although this hypothesis focused primarily on song quality and nutrition (Nowicki et al., 2002), it was later applied to broader contexts including other sexually selected traits (Spencer et al., 2011). We proposed a similar reasoning to explain why abandoners and accepters co-exist in some host populations, including yellow warblers (Sealy 1995; prediction 2a). However, feather CORT was not associated with the host's response to the model cowbird eggs (Figure 3.3). In addition, variance in feather CORT among the three treatment groups (control, accepted, abandoned) was similar (Figure 3.3). These results contrast with our predictions that control individuals, who did not experience the stress of have experimental parasitism, would display more variable stress histories and that experimental females would respond to parasitism according to their past stress histories (Bortolotti et al. 2008), thereby splitting the experimental parasitism groups into accepters and abandoners. Thus, feather CORT levels of female warblers did not predict responses to parasitism. This lack of an effect could occur if stress during moult was not high enough to induce carry-over effects (O'Connor et al., 2014). Overall, these results suggest that past stress, as measured through feather CORT, does not predict abandonment of parasitized clutches in yellow warblers within this study. Nonetheless, feather CORT only provides a proxy of CORT levels near the time of moult, and warblers may be responding to other time windows, such that additional study is warranted

Egg visual signals

The role of visual pattern differences in egg recognition has been widely established in other species, primarily in ejectors (Hanley et al., 2019; Honza and Cherry, 2017; Cassey et al. 2008; Spottiswoode and Stevens 2010). In response to increased egg recognition by hosts, some specialist brood parasites, such as common cuckoos, have evolved eggs that mimic the colour and maculation of their host (Davies and Brooke 1989a, b; Kilner 2006; Kilner and Langmore 2011; Spottiswoode and Stevens 2011; Thorogood et al. 2019). However, in generalist brood parasites such as brown-headed cowbirds, egg visual signals differ from hosts to a greater extent compared to specialist parasites such as cuckoos. As a result, visual signals may be more noticeable, and reduce the opportunity for coevolution of egg mimicry (Krüger 2007; Davies 2000). For instance, many cowbird hosts lay eggs that differ to varying degrees in colour and/or maculation from host eggs, like those of yellow warblers (Davies 2000). Warblers in our study had greater intra-egg maculation coverage and spot profile variation than the model cowbird egg (Figures 3 & 4), and females could have potentially used this egg visual signal as a cue to abandon their parasitized clutch. Indeed, yellow warblers can determine their clutch has been manipulated using both tactile and visual cues (Guigueno and Sealy 2012), but, prior to this study, the extent of differences in maculation of yellow warbler eggs were not quantitatively analyzed. Because we used model cowbird eggs, our comparison of warbler to cowbird eggs is limited. Experiments focusing on the response to natural cowbird eggs may be useful to further explore these results.

Visual recognition of parasitism via differences in maculation could modulate CORT changes. As such, we examined whether differences in maculation and spot profile variation between warbler eggs and our model cowbird eggs correlated with circulating CORT (prediction 3a, Figure 3.4). Increased differences in visual signals could be associated with increases in circulating CORT and decrease in parental care (Angelier et al. 2009). However, circulating CORT was not correlated with differences in maculation characteristics (Figure 3). Perhaps differences between cowbird and yellow warbler egg maculation were simply not large enough to stimulate a response. Indeed, some yellow warbler eggs look nearly identical to brown-headed cowbird eggs in maculation characteristics, and anti-parasitic behaviours in yellow warblers may be mediated through tactile, rather than visual, cues (Guigueno and Sealy 2009, 2012, 2014). Yet another possibility is that individuals that rejected experimental parasitism had higher baseline CORT, although we might still expect that abandonment would be associated with an interaction between CORT levels and visual signals.

Differences in egg maculation characteristics could be an important cue in eliciting egg rejection in brood-parasitic hosts, as has been shown in ejectors, but the same may hold true for clutch abandoners. Thus, we also examined whether differences in maculation characteristic variation predicted whether hosts accepted or abandoned their experimentally parasitized clutches (prediction 3b, Figure 3.5). Abandoners did not show greater variation in maculation coverage or spot profile between their eggs and the model cowbird eggs, relative to accepters, although the power of our analysis is limited by our small sample size for abandoners (n = 3; Figure 3.5). Although yellow warblers from a previous study gazed longer at manipulated clutches, this increased gaze time only occurred between eggs that differed in both background colour and maculation (i.e., blue vs off-white; brown maculation vs none; Guigueno and Sealy 2012). In addition, tactile cues, as measured by the warblers' egg probing and body shifting during incubation, had a stronger effect than visual cues (Guigueno and Sealy 2012). To abandon a clutch, females simply need to recognize they have been parasitized and use other cues such as tactile recognition of volumetric differences between the larger parasitic egg and the smaller host eggs (Guigueno and Sealy 2012) and interaction with the adult parasite (Guigueno and Sealy 2011). In sum, yellow warblers, as with other hosts that use clutch abandonment as an antiparasitic defense, seem to rely less on visual signals than egg-ejecting hosts to distinguish between their own eggs and the parasitic egg.

Conclusions & future research

We explored the effects of current (plasma) CORT, past (feathers) CORT, and differences in egg maculation variation in the context of clutch abandonment in parasitized yellow warblers. We found evidence that current stress, as approximated by circulating CORT in plasma, was associated with clutch abandonment, but we did not find that maculation or past stress played a role. To further explore the action of CORT, future studies could experimentally increase CORT to determine whether this manipulation elicits abandonment by female warblers. This approach would be opposite of that of Abolins-Abols & Hauber (2020)'s, in which authors decreased ejection in an American robin (*Turdus migratorius*) by experimentally decreasing CORT synthesis with mitotane injections. Robins normally eject at a high rate (i.e.,~90%; Croston and Hauber 2014), so decreasing CORT would result in more individuals accepting cowbird eggs. In contrast, yellow warblers normally abandon 20-30% of experimentally parasitized clutches, but up to about 44% of naturally parasitized clutches (Sealy 1995; Guigueno and Sealy 2011), possibly because females interact with the adult parasite (Guigueno and Sealy 2011). As such, in yellow warblers, increasing circulating corticosterone, via time-release pellets or silastic implants (Torres-Medina et al. 2018), for example, would potentially result in a larger number of abandoners than observed in our current study (i.e., a larger sample size) and would allow for causal, as opposed to correlational, conclusions. Experimentally increasing rates of abandonment through hormone manipulations would still reflect rates seen naturally, as cowbird parasitism and associated abandonment rates vary greatly geographically in yellow warblers (Kuehn 2009). In sum, baseline CORT was correlated with abandonment of experimentally parasitized clutches. Experimental manipulation of CORT levels would be needed to confirm a causal relationship.

In addition to manipulative studies, more research is needed to understand the role of other hormones and life history in mediating responses to brood parasitism. Hormones other than or in addition to CORT could influence clutch abandonment. Testosterone, progesterone, prolactin, and mesotocin may all play roles in abandonment of parasitized clutches, although this has primarily been examined in hosts that eject cowbird or cuckoo eggs, such as American robins and Eurasian blackbirds (*Turdus merula*), respectively (Abolins-Abols et al. 2018, Ruiz-Raya et al. 2020). In addition, stress is a mediator of life-history trade-offs (Bókony et al. 2009; Bonier et al. 2009; Abolins-Abols et al. 2018), and it would be valuable to incorporate parasitism status and sympatry with cowbirds in a large-scale analysis to examine interactions between hormones, life history traits, and brood parasitism. There is a great opportunity for future research to take a comprehensive endocrine approach in examining clutch abandonment, the costliest form of rejection in brood-parasitic hosts.

In conclusion, this study provides initial findings pertaining to the endocrine correlates of clutch abandonment in brood-parasitic hosts and confirms that egg visual signals play a less important role in eliciting abandonment than tactile cues. The yellow warbler-cowbird system provides a strong model to address mechanistic questions of clutch abandonment, and our research invites future experimental studies incorporating additional species that abandon clutches from diverse host-parasite systems. Our study, along with the proposed follow-up studies, will contribute to a comprehensive understanding of the intricate links between brood parasitism, hormones, and clutch abandonment, and the fitness-related trade-offs between incurring versus avoiding costs associated with brood parasitism and clutch abandonment.

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Ethics

This project was approved and conducted under the Banding Permit no. 23665, United States Fishes and Wildlife Service permit MB72878C-0 and Institutional Animal Care and Use Committee protocol # 19-05-02, all issued to S. A. Gill.

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CHAPTER 4: GENERAL DISCUSSION

In this thesis, I aimed to identify characteristics that would factor in the decision of hosts to abandon clutches parasitized by cowbirds (*Molothrus sp.*). I approached this question along two research axes. For the first, discussed in Chapter 2, I performed a phylogenetically controlled meta-analysis of published data of clutch abandonments by hosts of three best studied species of the cowbird clade. I then examined life history traits interactions that may explain the variation in clutch abandonment rate among hosts. In the second axis, covered in Chapter 3, I artificially parasitized yellow warblers (*Setophaga petechia*) with brown-headed cowbird (*Molothrus ater*) model eggs to examine relationships between clutch abandonment (egg burial or nest desertion) and mechanistic cues, namely corticosterone (CORT) levels and differences in maculation patterns between the host and model cowbird eggs.

Pursuing the meta-analysis and the experimental study on warblers allowed us to identify some interesting relationships. In general, clutch abandonment increases in response to parasitism. With the meta-analysis I found relationships between clutch abandonment and habitat, host sensitivity to parasitism, and migration. In the experiment on yellow warblers, I found that brood value played a role in predicting clutch abandonment in certain contexts, but overall appeared to be associated with populations that are either less exposed to parasitism or less sensitive to it. I found a relationship between clutch abandonment and circulating plasma CORT levels. All the factors mentioned above are important in explaining clutch abandonment rates. However, my results suggested that certain factors such as incubation time as investigated in the meta-analysis, and long-term past CORT and egg visual signals examined in the warbler experiment were not as significant in explaining clutch abandonment rates.

These results suggest that clutch abandonment is a central mechanism of modulation of parental care (Zuckerman et al. 2014), and is especially important in the context of brood parasitism. Studying the differences between different brood parasite hosts enables us to investigate what factors may be influencing clutch abandonment rates. Overall, I found that clutch abandonment was associated with a variety of factors relevant to the experience of a given individual, owing to their species' life history or to their own internal stress hormone levels, as discussed below.

Parasitism and clutch abandonment

As discussed in Chapter 2, overall I found clutch abandonment was consistently elevated in naturally parasitized individuals of a given population. This could be due to one or several aspects of brood parasitism, such as interaction with the parasitic egg, interaction with the adult cowbird, or social information from other individuals in the population (Davies 2000; Guigueno and Sealy 2012; Campobello et al. 2017). In Chapter 3, I found that brood parasitism as reproduced by model eggs did not appear to affect circulating yellow warbler CORT levels. Circulating glucocorticoids such as CORT are plastic and fluctuate in response to environmental stressors (Siegel 1980; Wingfield 2003). My results showed that simply adding model cowbird eggs, without the host abandoning the clutch, was not associated with an increase in circulating CORT (Figure 3.2a). This absence of a difference between controls and accepters, contrary to my prediction, is intriguing, as it suggests that individuals who did not abandon experienced a comparable level of stress compared to unparasitized (control) individuals. This finding differs from previous research; for instance, a common cuckoo (*Cuculus canorus*) host, the Eurasian blackbird (*Turdus merula*), experienced increased baseline CORT following experimental parasitism (Ruiz-Raya et al. 2018). The difference in findings could be dependent on several factors including parasitism rates, predation rates, breeding season length, and other life history traits (Bókony et al. 2009). The fact my study focused on a species relying on clutch abandonment, a variable abandonment response to parasitism, may explain the different pattern of circulating CORT variation that I observed, compared to species with more consistent responses such as Eurasian blackbirds which are egg ejectors (Ruiz-Raya et al. 2018).

Predictors of clutch abandonment

Brood value and clutch abandonment

It was predicted that brood value, as a quantification of the worth of a clutch as part of lifetime reproductive success for a given species, may contribute to explain the variation in clutch abandonment rates among different cowbird host species, as investigated in a meta-analysis in Chapter 2. While brood value alone did not explain clutch abandonment rates found in cowbird hosts on average, brood value explained clutch abandonment depending on parasitism and interactions with different life history factors.

Results were consistent in both habitat variables considered, i.e. forest/open and prairie/non-prairie. These variables represent a difference between a habitat of higher cowbird-host interaction (open and prairie) and a habitat with less regular or more recent cowbird exposure (forest and non-prairie). Species from higher cowbird-host interaction environments would be expected to be better adapted to respond to parasitism events (Mayfield 1965; Rothstein 1975). Meanwhile species breeding in more sheltered habitats are expected to encounter cowbirds less often and/or for shorter periods of time (Mayfield 1965; Smith et al. 2000). Therefore, the latter should be less responsive to parasitism, due to both reduced co-evolution pressure and individual experience (Soler et al. 2012; Blažek et al. 2018). For both habitat variables, brood value predicted clutch abandonment, but only in non-parasitized nests in species with lower cowbird-host interaction (i.e., non-prairie and forest habitats; figures 2.4 and 2.5, respectively). Brood value did not predict clutch abandonment for non-prairie and forest species when parasitized, while in host species that evolved with brood parasites (i.e., in open habitats and in prairies) brood value did not predict nest abandonment, regardless of the presence or absence of parasitism. My results suggested that species from habitats with higher cowbird exposure having to implement antiparasitic responses may have changed how brood value predicted clutch abandonment. In the absence of parasitism, birds may be more likely to abandon their nest if their brood value is low, following life history theory (Bókony et al. 2009). However, when parasitism comes into play, the effect of brood value is mitigated, and a general increase in the probability of clutch abandonment is observed. These results may suggest that the cost of parasitism is so high that when an individual is parasitized, brood value becomes irrelevant. In short, parasitism nullifies brood value.

I also predicted that some hosts would be more likely to abandon depending on host sensitivity. Sensitivity is defined as the size of the fitness cost they incur from being parasitized. I examined two variables as proxies for the cost of parasitism: body mass and incubation time (Hosoi and Rothstein 2000). High body mass and short incubation time were both associated with low sensitivity to parasitism, while low body mass and longer incubation time corresponded to high sensitivity. When parasitized, large prairie species exhibited a trend of increasing abandonment with decreasing brood value (Figure 2.10). This relationship was only observed in this particular set of conditions. This negative relationship between brood value and clutch abandonment was consistent with the direction of my predictions, however its presence only in large species and in the prairie habitat is not. In line with my observations that brood value did not predict abandonment in species from habitats with higher cowbird exposure, species with a higher host sensitivity may exhibit a comparable effect, whereby the costs of parasitism are so high that the importance of brood value is mitigated, while an effect of brood value would be visible in the group with the least sensitivity to parasitism. Less expected is this relationship being present only in the prairie group, the higher cowbird exposure habitat, as opposed to the non-prairie group. This differs from the habitat-only analyses, where brood value was not important in species more exposed to parasitism.

Migratory species were predicted to have a higher brood value associated with decreased clutch abandonment, following my prediction that migration puts constraints on breeding (Zink 2011). I found that migrants had a higher brood value than non-migrants, in line with my predictions. Migratory species also had a lower clutch abandonment rate than non-migrants (Figure2.11), suggesting they may be under more pressure to not abandon, which may be due to increased constraints put on breeding by migration (Zink 2011). However, there was no relationship between clutch abandonment and brood value when accounting for migratory status (Figure 2.12). There may be additional factors underlying the links between brood value, migration, and clutch abandonment, such as costs of migration not accounted for in my analyses, such as survival or the physical demands of migration (Zink 2011).

Overall, my results suggest that brood value predicts abandonment in some conditions. The relationship is present in the absence of parasitism in low cowbird-host interaction species, suggesting parasitism overrides brood value. This link between clutch abandonment and brood value is also observed in some conditions of lowered host sensitivity where abandonment rates are also generally lower, while depending on complex interactions of habitat and sensitivity factors. Finally, my results indicate migration is linked with increased brood value, but this effect is not associated with an effect of brood value on clutch abandonment, suggesting different factors are driving the variation.

Circulating plasma corticosterone and abandonment

Increased circulating CORT was predicted to be an important factor associated with clutch abandonment (Landys et al. 2006; Scharf et al. 2021). In my study of experimentally parasitized yellow warblers, as described in Chapter 3, females who abandoned their parasitized clutch had elevated circulating CORT, relative to control clutches that were not parasitized

(Figure 3.2a). This suggests that abandoners in the population had a more responsive endocrine response than accepters, whose circulating CORT did not increase in response to the same stressor. Alternatively, the process of abandoning the clutch itself, either by egg burial or nest desertion, could have increased circulating CORT. Elevated circulating CORT in abandoners could be associated with increased survival-related behaviours, potentially at the expense of the current reproductive effort, such as increased vigilance and suppressed parental behaviours (Voellmy et al. 2014). Given that clutch abandonment is a generalized response, it is likely that similar increases in circulating CORT occur in response to other abandonment-triggering factors, such as inclement weather (Romero et al. 2000; Thierry et al. 2013). Therefore, circulating CORT could provide us with a broader understanding of how environmental stressors, rather they be biotic or abiotic, affect nest abandonment in birds. My results were in line with the findings of Abolins-Abols and Hauber (2020) who found a causal link between stress-induced CORT and egg ejection rate in American robins, an egg-ejector species. Together, these findings suggest baseline CORT may mediate antiparasitic responses across different parasite-host systems and across rejection methods (Ibáñez-Álamo et al. 2012; Mark and Rubenstein 2013; Schoenle et al. 2017).

Habitat and clutch abandonment

I predicted that host species that are more in contact with parasites will be more responsive. All species of cowbirds included in this study are mostly prairie species whose range are expanding due to habitat transformation (Mayfield 1965; Smith et al. 2000). When considering habitat and the effect of parasitism alone not taking brood value into account, we found that while parasitism increases clutch abandonment in all cases, clutch abandonment was lower in forested habitats compared to open habitats, which, again, are higher cowbird-host interaction habitats (Figure 2.3). Past research has found a similar relationship when comparing parasitism rates between open and forested habitats (Hosoi and Rothstein 2000). This difference has been suggested to be evidence of evolutionary lag between species that encounter parasitism more often and those whose harder to access breeding habitat shields them to some extent from more frequent parasitism. My results, using a larger dataset and more species of parasites than previous studies, reinforced their findings regarding habitat and parasitism response and suggest that species with higher parasite-host interactions have more responsive defenses to parasitism (Hosoi and Rothstein 2000).

Host sensitivity and clutch abandonment

As mentioned above, I examined two variables for host sensitivity: body mass and incubation time. High body mass and short incubation time were both associated with low sensitivity to parasitism, while low body mass and longer incubation time corresponded to high sensitivity. Body mass had an effect under certain conditions. Large body mass (i.e. low sensitivity) species were less likely to abandon their clutch in general when compared to small (i.e. high sensitivity) species, regardless of parasitism (Figure 2.7). While the difference was as predicted in that higher sensitivity species showed increased abandonment rates, the lack of relation to parasitism diverges from my predictions by suggesting that parasitism is not a driver of this difference. The observed greater propensity of small species to abandon in general may suggest that they are more vulnerable to factors impacting breeding in general, such as weather or predation (Robin et al. 2001).

Possible non-predictors of clutch abandonment

While I was able to identify the aforementioned factors as important factors in explaining how clutch abandonment varies between individuals and species, I also found some factors for which no relationship was identified in this context. These are informative as they help identify how some factors may not be as important in explaining clutch abandonment rates.

Incubation time could be expected to influence host sensitivity to parasitism, and the magnitude of antiparasitic responses as a result. The relatively short incubation time of cowbirds relative to certain hosts may increase the cost of parasitism experienced (Briskie and Sealy 1990). While body mass, the other variable considered to measure host sensitivity, explained clutch abandonment differences, incubation time did not explain clutch abandonment variation (Figure 2.6), which suggests incubation time is not central of a factor in explaining differences in response to parasitism.

Feather CORT, in contrast to circulating plasma CORT, indicates long-term past stress history (i.e., at the time of moult; Bortolotti et al. 2008). As such, I predicted that abandoners and accepters would have higher and lower past CORT than controls, respectively, focused on individuals' prior state to determine whether it could predict a response to the future stressor of experimental parasitism. As such, these predictions resemble those of the developmental stress hypothesis, which proposes that stress early in life (e.g., nestling nutritional state) can affect behaviour and cognition later in the life (e.g., adult song quality). I proposed a similar reasoning to explain why abandoners and accepters co-exist in some host populations, including yellow warblers (Sealy 1995). However, my data showed no evidence that feather CORT was associated with the host's response to the model cowbird eggs (Figure 3.3). In addition, variance among the three treatment groups (control, accepted, abandoned) was similar. This is in contrast with my prediction that control individuals, who did not experience the stress of having model cowbird eggs added to their nests, would represent individuals with variable stress histories within the study population. I further predicted that individuals who experienced the addition of model cowbird eggs would respond according to their past stress histories (Bortolotti et al. 2008) and split the experimental parasitism groups into accepters and abandoners. I found no support for these predictions, indicating that feather CORT levels do not predict response to parasitism. Overall, these results suggest that past stress, as measured through feather CORT, does not predict abandonment of parasitized clutches in yellow warblers within this study.

The role of visual pattern differences in egg recognition, which play a role in the ability to respond to parasitism, has been widely established in other species, primarily ejector species (Cassey et al. 2008; Spottiswoode and Stevens 2010; Honza and Cherry 2017; Hanley et al. 2019). However, in generalist brood parasites, such as brown-headed cowbirds, egg visual signals differ from hosts to a greater extent as cowbirds show less host specialization than cuckoos, and thus, may be more noticeable, and less of an opportunity for coevolution of egg mimicry (Davies 2000; Krüger 2007). Increased differences in visual signals could be associated with increases in circulating CORT and decrease in parental care (Angelier et al. 2009). However, circulating CORT was not significantly correlated with differences in maculation characteristics (Figure 3.4 and 3.5). Perhaps variation between cowbird and yellow warbler egg maculation is simply not large enough to stimulate increased vigilance. Indeed, some yellow warbler eggs look nearly identical to brown-headed cowbird eggs in maculation characteristics (Guigueno and Sealy 2009). Alternatively, antiparasitic responses to egg visual signals in yellow warblers may rely more on other factors, such as tactile cues, which may be mediated by CORT (Guigueno and Sealy 2012; Guigueno et al. 2014).

Additionally, I aimed to determine whether differences in maculation characteristic variation could predict whether hosts accepted or abandoned their experimentally parasitized clutch. However, abandoners did not show greater variation in maculation coverage or spot profile between their eggs and the model cowbird eggs, relative to accepters. Yellow warblers, as may be the case with other hosts that use clutch abandonment as an antiparasitic defense, seem to rely less on visual signals than egg-ejecting hosts to distinguish between their own eggs and the parasitic egg and subsequently reject their parasitized clutch (Guigueno and Sealy 2011, 2012). Hosts that abandon may not need to visually recognize their own eggs or identify a parasitic egg since the abandonment of the clutch discards all eggs, including their own.

Conclusions, implications and further questions

Overall, this thesis utilized a two-pronged approach to the study of clutch abandonment in the context of brood parasitism, by encompassing both a broad dataset encompassing hundreds of cowbirds hosts to explore the importance of life history traits in antiparasitic responses, and a focused study to identify mechanistic cues in yellow warblers, a frequent clutch abandoning host of the brown-headed cowbird. As a result, I identified several interrelated factors that can explain variation in clutch abandonment differences at the species and individual level.

In my meta-analysis, brood value was shown to be an important variable to consider to explain differences in clutch abandonment, but brood parasitism has a greater and potentially overriding effect compared to brood value when it comes into play, both at the species and individual level. Species with lower sensitivity to parasitism similarly show an impact of brood value, as complex interaction suggested again that parasitism nullifies brood value in species with a higher host sensitivity. These results offer possible avenues to understand broad life history factors and how they explain variation in host tolerance of brood parasitism, all the while providing insight in how brood value may inform parental care in a context of lowered fitness.

My findings on life history traits may be interpreted in different ways in the context of explaining the importance of lifetime reproductive success in determining abandonment rates. Under the evolutionary lag hypothesis, host tolerance of parasitism is explained as being primarily the result of a failure to recognize parasitism (Underwood and Sealy 2006). As discussed earlier, the increase of clutch abandonment associated with increased cowbird-host interaction may be supportive of the presence of an evolutionary lag (Hosoi and Rothstein 2000), and parasitism negating brood value may be seen as further evidence that the cost of parasitism precludes any other response than abandonment. However, the lack of effect of brood value in the presence of parasitism may be the result of trade-offs not quantified by brood value mediating the relationship. Breeding individuals also face pressures at the scale of the breeding season rather than the individual's entire lifetime. Notably, the specific timing of clutch onset during a breeding season is a relevant factor, as the cost of clutch abandonment compared to the cost of parasitism acceptation can change over the course of the season (Sealy 1995; Guigueno and Sealy 2010). Indeed, towards the end of a breeding season, conditions rapidly become less favorable for breeding. As a result, the cost of abandonment and delayed renesting can grow larger than the fitness cost of accepting parasitism (Guigueno and Sealy 2010). The likelihood of abandonment has also been shown to increase greatly when a cowbird lays their egg before the host lays (Sealy 1995). In the context of this study, these short-term timing effects were not included because of the amalgamation of multiple studies, including studies conducted over multiple seasons or lacking clutch onset data. Therefore, while my results can provide support for the evolutionary lag hypothesis, they do not disprove the evolutionary equilibrium hypothesis, as the variation in clutch abandonment could be mediated by factors not taken into account by brood value. Taking the timing of parasitism compared to host laying into account in further research on hosts' antiparasitic response may be valuable to understand how changes throughout the season influence differences in clutch abandonment rates.

From my experimental study of yellow warblers, I found evidence that current stress, as measured by circulating CORT in plasma, was associated with abandonment. I did not find evidence that visual signal or past stress played a role. However, recall that the small samples sizes limit statistical power. Sample sizes were smaller for abandoners than for other treatments, and sample sizes were overall lower for feather CORT ($n = 29$) and egg visual signal ($n = 15$) analyses compared to circulating CORT analyses ($n = 40$; Table 3.1). To further explore the action of CORT, future studies could experimentally increase CORT to determine whether this manipulation would elicit abandonment in individuals that would not have otherwise. In yellow warblers, increasing circulating CORT, via time-release pellets or silastic implants (Torres-Medina et al. 2018), for example, would potentially result in a larger number of abandoners than observed in my current study (i.e., a larger sample size) and would allow for causal, as opposed to correlational, conclusions. Experimentally increasing rates of abandonment would still reflect rates seen in non-experimental settings, as cowbird parasitism and associated abandonment rates vary greatly geographically in yellow warblers (Kuehn 2009). In sum, I can only conclude that circulating CORT was associated with abandonment of experimentally parasitized clutches, and not caused it. Finally, hormones other than CORT could have an effect on clutch abandonment. Hormones such as testosterone, progesterone, prolactin, and mesotocin may all play roles in abandonment of parasitized clutches, although this has primarily been examined in hosts that eject cowbird or cuckoo eggs, such as American robins and Eurasian blackbirds (*Turdus merula*), respectively (Abolins-Abols and Hauber 2018; Ruiz-Raya et al. 2020). There is a great opportunity for future research to take a comprehensive endocrine approach in examining the costliest form of rejection in brood-parasitic hosts, clutch abandonment.

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My experimental study provides initial findings into the endocrine effects of clutch abandonment in brood-parasitic hosts and confirms that egg visual signals play a less important role. The yellow warbler-cowbird system is a strong model to address mechanistic questions of clutch abandonment, and my research invites future experimental studies incorporating several clutch abandoners from diverse host-parasite systems. Furthermore, research into differences in clutch abandonment response incorporating clutch onset timing factors may be valuable in the future. My study, along with the proposed follow-up studies, would provide a comprehensive understanding of the intricate links between brood parasitism, hormones, and clutch abandonment, and the fitness-related trade-offs between incurring versus avoiding the costs associated with brood parasitism and clutch abandonment.

Stress may be a mediator of life-history trade-offs (Bókony et al. 2009). Baseline CORT was shown to be positively correlated with brood value in an avian meta-analysis (Bókony et al. 2009). Furthermore, species with a higher brood value had a lowered stress response, presumed to improve their reproductive success by mitigating the negative effect of glucocorticoids like CORT on parental care (Bókony et al. 2009). This study of a relationship between life history traits and stress response is what links together Chapters 2 and 3 of my thesis, as I approached the topic of clutch abandonment in the context of brood parasitism from both an endocrine perspective and a life history perspective. The differences in clutch abandonment rates observed in my meta-analysis of life history traits may be mediated by endocrine changes such as those observed in my experimental study. My study of life history traits and their link to clutch abandonment is suggestive of a concrete link between them. In conclusion, this thesis reaffirms that brood parasitism constitutes a strong and diverse model to study the mechanisms of clutch abandonment and more broadly, parental care.

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