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Factors Affecting The Expression of the Dear Enemy Effect in Territorial Songbirds

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**FACTORS AFFECTING THE EXPRESSION OF THE DEAR ENEMY EFFECT IN
TERRITORIAL SONGBIRDS**

by

CHRISTOPHER MOSER-PURDY

A Thesis

Submitted to the Faculty of Graduate Studies through Biological Sciences in
Partial Fulfillment of the Requirements for the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2016

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Factors Affecting the Expression of the Dear Enemy Effect in Territorial Songbirds

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Declaration of Co-Authorship/Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is the result of joint research as follows:

I am the sole author of chapters 1 and 5, and the principal author of Chapters 2, 3, and 4. Chapters 2, 3, and 4 were conducted under the supervision of Daniel Mennill who contributed input on the experimental design, analyses, and writing, as well as providing funding for the field research. Chapter 3 was also conducted with the help of Elizabeth MacDougall-Shackleton who contributed through assistance with field work and input on analyses and writing. Chapter 4 was conducted with the help of Scott MacDougall-Shackleton who contributed input on lab work, analyses, and writing; Frances Bonier who contributed input on experimental design, analyses, and writing; Andrea Boyer who assisted with lab work, analyses, and writing; and Brendan Graham, who contributed input with field work, and with experimental design, analyses, and writing. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by myself, with input from my collaborators.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis.

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II. Declaration of Previous Publication

This thesis includes one original paper that has been published in a peer-reviewed journal, as follows:

Thesis Chapter	Publication title/full citation	Publication Status
Chapter 2	Moser-Purdy, C., & Mennill, D.J. (2016). Large vocal repertoires do not constrain the dear enemy effect: a playback experiment and comparative study of songbirds. <i>Anim. Behav.</i> ANBEH A16-00035R	Published: <i>Animal Behaviour</i> , 118, 55–64.

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Abstract

Many territorial animals respond more intensely to strangers than neighbours. This phenomenon is known as “the dear enemy effect”. This phenomenon occurs because strangers represent a threat to territory takeover and parentage whereas neighbours only represent a threat to parentage. Many studies have investigated whether diverse animals exhibit the dear enemy effect, but few have examined the underlying factors that mediate this phenomenon. I tested whether three factors – male repertoire size, female fertility status, and male testosterone levels – influence the dear enemy effect in male songbirds. I found that repertoire size had no effect on dear enemy effect expression; that female fertility status influenced flexibility in dear enemy effect expression over a breeding season; and that testosterone does not seem to be associated with dear enemy effect expression. Overall these results show that several factors influence the dear enemy effect and that the dear enemy effect is a dynamic phenomenon.

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CHAPTER 1

GENERAL INTRODUCTION

Introduction

Animals use acoustic signals for territory defense and mate attraction (Bradbury & Vehrencamp, 2011). These acoustic signals usually have fine structural differences between individuals that allow animals to distinguish between different individuals (e.g. Kirschel et al. 2011; Osiejuk, 2014; Wilson & Mennill, 2010). When an animal hears a signal from a conspecific individual it must decide how to respond; an aggressive individual would naturally require a different response than a non-aggressive individual. One important distinction that territorial animals must make on a regular basis is that between conspecific neighbours and strangers. Neighbours – animals living in a territory adjacent to an individual – are familiar individuals that an animal should have regular interactions with (Getty, 1987). Strangers – animals that an individual has had no contact with – are unfamiliar individuals (Getty, 1987). My thesis investigates the factors that influence and drive responses to neighbours versus strangers. In this general introduction I will provide an overview of the field of neighbour-stranger discrimination and discuss three factors that may influence neighbour-stranger discrimination. I will also introduce my study species and the study site where I conducted my research.

Neighbour-Stranger Discrimination and the Dear Enemy Effect

During the breeding season, diverse animals form and defend multi-purpose breeding territories (Temeles, 1994). In the North Temperate Zone, males are usually

the defenders of these multi-purpose territories which are used for attracting mates, foraging, and raising young (Temeles, 1994). Territories are essential for the reproductive success of a male; few non-territorial males are ever the sires of offspring in socially monogamous territorial species (Hill et al., 2011; Griffith et al. 2002; Mennill et al. 2004). Thus, securing and retaining a territory are of paramount importance to males.

There are two broad classes of conspecific individuals a territorial animal may encounter: familiar and unfamiliar (Stoddard, 1996). Neighbours and strangers present a dichotomy between these two classes since both are rival males but neighbours are familiar whereas strangers are unfamiliar. In the wild, strangers are often “floater” birds without territories of their own, thus they represent a threat to an animal’s territory ownership (Getty, 1987). Conversely, neighbours, who by definition already hold a territory, do not represent a great threat to an animal’s territory ownership (Getty, 1987). However, both male neighbours and strangers represent a threat to a male animal’s paternity through copulation with his partner during her fertile period (Getty, 1987; Temeles, 1994). Taken together, strangers should be a greater threat because they represent a threat to an animal’s territory and paternity while neighbours represent a threat only to an animal’s paternity (Temeles, 1994).

Since neighbours and strangers represent different levels of threat, it benefits territorial animals to be able to discriminate between these classes of conspecific individuals. Animals can gain fitness benefits by spending more time foraging or caring for young instead of engaging in costly territorial disputes with low-threat rivals. Indeed,

neighbour-stranger discrimination has been documented across a wide array of taxa including passerine birds (e.g. Draganoiu et al., 2014), non-passerine birds (e.g. Hardouin et al., 2006), amphibians (e.g. Feng et al., 2009), mammals (e.g. Randall, 1989), lizards (e.g. Whiting, 1999), insects (e.g. Newey et al., 2010), crustaceans (e.g. Booksmythe et al., 2010), and fish (e.g. McGregor & Westby, 1992). Most studies report an increase in aggression toward strangers versus neighbours.

An increased response to strangers versus neighbours is known as the “dear enemy effect”, a term originally coined by James Fisher in 1954. Weeden and Falls (1959) conducted the first playback experiment that investigated the dear enemy effect, using Ovenbirds (*Seiurus aurocapilla*) as their study species. This experiment consisted of presenting individual Ovenbirds with recorded songs of a neighbour and recorded songs of a stranger then observing the responses of each Ovenbird. If their birds responded more aggressively to stranger playback (e.g. approached closer to the loudspeaker, spent more time near the loudspeaker) this would support the idea that Ovenbirds perceived strangers as a greater threat than neighbours, and therefore provide evidence of the dear enemy effect. Indeed, this is what they found (Weeden & Falls, 1959). Since this pioneering study, researchers have conducted over a hundred studies (see Stoddard 1996 and Temeles 1994 for early reviews) on neighbour-stranger discrimination and the dear enemy effect, largely finding similar results to Weeden and Falls (1959).

Expressing the dear enemy effect allows animals to engage in costly territorial disputes only when faced with a high threat individual and expend less energy on aggressive displays when faced with a low threat individual. Importantly, animals are

responding to the threat level of an individual, rather than the familiarity. In some species, neighbours may be an increased threat due to mate switching (e.g. Winter Wrens, *T. troglodytes*, Courvoisier et al., 2014), decrease in nesting site space such as in colonially nesting seabirds (e.g. Laughing Gulls, *Leucophaeus atricilla*; Beer, 1970) or a threat to territory size in group-living animals (e.g. banded mongooses, *Mungos mungo*; Müller & Manser, 2007). The dear enemy effect is a general trend seen across most species (Temeles, 1994). However, it is important to bear in mind that threat-level is the driver of this phenomenon, instead of familiarity.

The majority of dear enemy effect studies aim to determine if animals display the dear enemy effect through behavioural response measures. From this research, we have a good understanding of which species do or do not display the dear enemy effect and we have some understanding of the social environment and threat-levels of conspecific individuals in different species. However, despite the extensive study on expression of the dear enemy effect, few studies have investigated the underlying processes or factors that may influence dear enemy effect expression. Three of these factors which I will discuss are song repertoire size, female fertility status, and testosterone.

Repertoire Size

Many birds use vocalizations to communicate with conspecific individuals. Passerines of the parvorder Passerida (i.e. songbirds) use songs to communicate. Songs are complex acoustic signals and are largely used for mate attraction and territory

defense (Catchpole & Slater, 2008). Many songbirds produce multiple songs that comprise a song repertoire, ranging from two song types to thousands of song types (MacDougall-Shackleton, 1997). Large song repertoires may have evolved to allow more complex communication between conspecific individuals through the use of song matching or repertoire matching (Beecher et al., 1996) and as an indication of male quality for female mate choice (e.g. Hesler et al., 2012; Searcy & Marler, 1981).

Early research on neighbour-stranger discrimination provided evidence that large song repertoires may impose a constraint that inhibits the ability of birds to recognize their neighbours (e.g. Godard, 1993; Kroodsma, 1976; Searcy et al., 1981). Larger repertoires may constrain recognition because a bird would need to learn more songs to recognize its neighbours and would hear each song a fewer number of times as a bird cycled through its repertoire, giving the neighbour less of a chance to memorize each song (Stoddard, 1996). Falls and D'Agincourt (1981) investigated neighbour-stranger discrimination in two closely related species with differing repertoire sizes: the large-repertoire Eastern Meadowlark (*Sturnella magna*), and the small-repertoire Western Meadowlark (*Sturnella neglecta*). They found strong neighbour-stranger discrimination in Western Meadowlarks but no neighbour-stranger discrimination in Eastern Meadowlarks, suggesting that repertoire size may inhibit discrimination in Eastern Meadowlarks. However, evidence exists that contradicts this hypothesis, with large repertoire birds such as the European Robin (*Erithacus rubecula*) differentiating between neighbours and strangers (Brindley, 1991) and a comparative analysis of 20 songbirds that shows no effect of repertoire size on a bird's ability to recognize neighbours (Weary

et al., 1992). Still, some question still exists as to whether large song repertoires constrain neighbour-stranger discrimination. This open question was the motivation for the first data chapter in this thesis (Chapter 2).

Fertility Status

Many birds form socially monogamous pair bonds, within which a male and a female will inhabit a territory and raise young together during a breeding season (Hasselquist & Sherman, 2001). Throughout the breeding season, birds will pass through several breeding stages which include periods of female fertility and periods when the female is not fertile. Female fertility status has been shown to affect multiple behaviours including singing behaviour (e.g. Ballentine et al., 2003; Zhang et al., 2016) and mate guarding behaviour (e.g. Hamao, 2000; Mass, 2009). A reason for these changes in behaviour is that although most birds are socially monogamous, the overwhelming majority of birds (86%) are genetically promiscuous (Griffith et al., 2002). Thus, a male may alter behaviour during the fertile periods of his mate and his neighbours' mates in order to protect his own paternity and to sire extra-pair offspring.

In order to maximize his reproductive success, a male will benefit by protecting his paternity. Neighbours and strangers both theoretically represent a threat to a male's paternity, although neighbours may be an increased threat to paternity because neighbours are often the sires of extra-pair young (Griffith et al., 2002; Hill et al., 2011; Mennill et al., 2004). Therefore, it may benefit a male to respond more aggressively to a

neighbour during periods when his female is fertile than periods when his female is not fertile.

Previous studies have found that the dear enemy effect is flexible over different breeding stages (Briefer et al., 2008; Courvoisier et al., 2014) and times of year (Hyman, 2005). For example, Briefer et al. (2008) found that Skylarks (*Alauda arvensis*) displayed the dear enemy effect in the middle of the breeding season but not at the beginning or end of a breeding season. These three studies observed changes in the dear enemy effect over different time periods, however, they did not directly investigate the association of their study subjects' responses with female fertility or any other factors. Thus, the reasons for the observed flexibility in the dear enemy effect are poorly understood. It is likely that there are multiple factors that contribute to this flexibility, however fertility status is likely one of the most important due to the increased threat neighbours pose to an individual's reproductive success during fertile periods. This idea is the motivation for the second data chapter of my thesis (Chapter 3).

Testosterone

Hormones play an important role in the stimulation and regulation of physiological functions and behaviours. Testosterone is a steroid hormone that has been associated with increased aggression (e.g. Cavigelli et al., 2000; Hau et al., 2000; Mougeot et al., 2005), increased mating success (e.g. Augustine et al., 2011; Smith et al., 2015), and increased territory size (e.g. Alonso-Alvarez & Velando, 2001; Chandler et al.,

1994). Despite these positive effects of testosterone, it has also been associated with decreased nestling provisioning and paternal care (e.g. Lynn et al., 2009; Peters et al., 2002) and may have detrimental effects on the immune system (e.g. Fargallo et al., 2007). Consequently, it may benefit an animal to increase testosterone levels during periods when increased aggression is needed, but keep testosterone levels low during periods where decreased aggression is preferred in order to reduce the detrimental effects of testosterone.

Under the Challenge Hypothesis, territorial male animals should increase their testosterone levels during periods of social instability such as during a challenge from a conspecific male (Wingfield et al., 1990). Indeed, in many species, males have increased testosterone in the early breeding season, during territory establishment when territory border negotiations are commonplace (e.g. Landys et al., 2010; Wingfield & Hahn, 1994). This is followed by a decrease in testosterone levels later in the breeding season when territories are established and parental care is needed (Wingfield et al., 1990). Furthermore, some male animals show increased testosterone levels after a simulated territorial intrusion (e.g. Desjardins et al., 2006; Wikelski et al., 1999), although this is not always the case (e.g. Deviche et al. 2012; Rosvall et al. 2012). Since testosterone may be used to mediate responses to territorial challenges, it stands to reason that individuals will increase testosterone levels more when confronting a stranger than when confronting a neighbour. In this way, testosterone may mediate the behavioural responses observed in dear enemy effect studies. Only one study has previously examined this idea and found an increase in 11-ketotestosterone upon exposure to

strangers versus exposure to neighbours in a fish species (Aires et al., 2015). Although a large body of work exists on the behavioural responses associated with the dear enemy effect, little work has been conducted on the physiological responses that may mediate this phenomenon. This idea is the motivation for the third data chapter of my thesis (Chapter 4).

Study Site and Species

I conducted field work at the Queen's University Biological Station (44° 34' N, 76° 19' W) north of Kingston, Ontario, Canada. This study site contains temperate forests interspersed with marshes and wetlands that provide a habitat for a wide variety of songbirds including my two study species: Red-eyed Vireos (*Vireo olivaceus*) and Song Sparrows (*Melospiza melodia*).

Red-eyed Vireos

Red-eyed Vireos are temperate-breeding songbirds that usually inhabit deciduous or deciduous-coniferous forests (Cimprich et al., 2000). Males are roughly 21 grams and their diet mainly consists of insects (Cimprich et al., 2000). They have large song repertoires (31 song types, Borror, 1981; 59 song types, Godard, 1993) and their songs have a whistle-like tone and are comprised of between one and five short syllables with songs averaging 0.3 seconds in length (Borror, 1981). Red-eyed Vireos are prolific

singers, averaging roughly 43 songs sung per minute during the breeding season (Borror, 1981) and frequently sing from shortly before dawn until late afternoon (Cimprich et al., 2000). Red-eyed Vireos nest high in trees with open cup nests and usually have one brood per breeding season (Cimprich et al., 2000). At my study site, Red-eyed Vireos inhabit forest territories at a high density.

Godard (1993) investigated if Red-eyed Vireos were capable of discrimination between individual neighbours and found results that suggested that Red-eyed Vireos were incapable of this task or had difficulties discriminating among conspecific neighbours. Due to their large repertoire size, this study provided support for the idea that repertoire size constrains conspecific discrimination. The conclusions of this study have been called into question because a lack of behavioural differentiation does not necessarily indicate a lack of recognition (Stoddard, 1996). Importantly, although Red-eyed Vireos may have difficulty with neighbour-neighbour discrimination, they may not have as much difficulty with neighbour-stranger discrimination; the former involves differentiating between two familiar individuals while the latter involves differentiating between one familiar and one unfamiliar individual (Stoddard, 1996). Although previous research has found a lack of discrimination in Red-eyed Vireos, more research is necessary to determine the extent of their discrimination abilities.

Red-eyed Vireos employ a mixed reproductive strategy, with 57% of broods having at least one extra-pair offspring in a Pennsylvanian population (Morton et al., 1998). Thus in this species it is likely that neighbours represent a high threat to male parentage during periods of female fertility. Male Red-eyed Vireos may benefit from

increased mate guarding and aggression toward intruding neighbours during their partner's fertile period.

Song Sparrows

Song Sparrows are temperate breeding songbirds. They weigh 25 grams although there is a large range in mass depending on the location within their distribution where they are sampled (23-28 grams; Arcese et al., 2002). Song Sparrows usually inhabit marshes, swamps, or fields (Nice, 1943). They nest in a wide variety of locations including junipers, trees, cattails, and on the ground, and usually have two broods (Nice, 1943). Their nests are composed of dead grass and reeds (Nice, 1943). They have medium-sized song repertoires (9.6 song types; Wilson et al., 2000). Their songs are complex and are comprised of multiple different parts with different qualities including trills, whistles, and buzzes (Arcese et al., 2002).

Song Sparrows have been subject to multiple neighbour-stranger discrimination experiments. Early experiments showed weak neighbour-stranger differentiation in Song Sparrows, which led researchers to believe this was due to their medium sized repertoire (Harris & Lemon, 1976; Kroodsma, 1976; Searcy et al., 1981). A carefully designed study by Stoddard et al. (1990), however, showed that Song Sparrows were capable of strongly discriminating between neighbours and strangers and that they unambiguously displayed the dear enemy effect. Further studies demonstrated that Song Sparrows also differentiate between neighbours based on threat-level (Akçay et al.,

2009). Song Sparrows readily discriminate between conspecific individuals and display the dear enemy effect.

Like Red-eyed Vireos, Song Sparrows also employ a mixed reproductive strategy. Multiple studies have reported high levels of extra-pair paternity in different Song Sparrow populations (e.g. 36.1 % of broods, Hill et al. 2011; 44.0% of broods Sardell et al., 2010), with most of these extra-pair fertilizations from neighbouring males. Thus, neighbours are likely a high threat to males during the fertile periods of females, and male Song Sparrows may benefit from increased aggression toward non-cooperative neighbours during this time period.

Multiple studies have explored the effects of testosterone in Song Sparrows (reviewed in Soma, 2006). Testosterone levels are highest during territory establishment in male Song Sparrows, when aggressive interactions between neighbours are common (Wingfield, 1984a). Furthermore, testosterone is known to be associated with aggressive responses to simulated territorial intrusions in Song Sparrows (Wingfield & Wada, 1989) and testosterone implants lead to an increase in aggressive behaviours in Song Sparrows (Wingfield, 1984b). Testosterone implants also lead to an increased territory size and polygyny in the usually socially monogamous Song Sparrow (Wingfield, 1984c). Conversely, increased testosterone levels have been shown to decrease fat stores in Song Sparrows which may indicate an increased energetic cost of high testosterone levels (Wingfield, 1984c). The effect of testosterone in Song Sparrows is fairly well understood and it is clear that testosterone plays a role in regulating aggression in this species.

Thesis Goals

The goal of my thesis is to investigate how different factors can affect and influence expression of the dear enemy effect. In Chapter 2, my goal is to investigate if repertoire size inhibits or constrains neighbour-stranger discrimination in Red-eyed Vireos as well as across multiple songbird species. In Chapter 3, my goal is to investigate if female fertility status affects the expression of the dear enemy effect in Song Sparrows. In Chapter 4, my goal is to investigate if Song Sparrows have increased testosterone levels when exposed to stranger playback versus when they are exposed to neighbour playback. Many studies have examined whether animals are capable of displaying the dear enemy effect but few studies have examined what factors influence neighbour-stranger discrimination or dear enemy effect expression. A more comprehensive understanding of the factors at play when animals are faced with conspecific neighbours versus strangers gives us increased knowledge regarding the social interactions and territorial dynamics in territorial species.

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CHAPTER 2

LARGE VOCAL REPERTOIRES DO NOT CONSTRAIN THE DEAR ENEMY EFFECT: A PLAYBACK EXPERIMENT AND COMPARATIVE STUDY OF SONGBIRDS

Chapter Summary

Many territorial animals are less aggressive towards neighbours than they are towards strangers. This phenomenon is known as the 'dear enemy' effect and it occurs because strangers represent a considerably higher threat to territory take-over compared to neighbours. Some evidence has suggested that large repertoires may constrain neighbour–stranger discrimination. We tested whether songbirds with large repertoires exhibit neighbour–stranger discrimination, conducting a playback study on a songbird with a large vocal repertoire, and a comparative analysis of the dear enemy effect across all published studies of songbirds. In our playback study, we broadcast neighbour and stranger songs within the breeding territories of Red-eyed Vireos (*Vireo olivaceus*) a songbird species with a large song repertoire (ca. 50 songs per individual). Vireos responded significantly more aggressively to playback of stranger versus neighbour songs; subjects approached closer to the loudspeaker, had a lower latency to approach the loudspeaker, spent more time near the loudspeaker and sang more soft songs during stranger trials than during neighbour trials. We examined song sharing between Red-eyed Vireos and found low levels of song sharing between neighbours, suggesting that Red-eyed Vireos may discriminate among conspecifics based on individually distinctive song types. We then conducted a comparative analysis of neighbour–stranger discrimination across the published literature on songbirds, using a phylogenetically controlled analysis to explore whether species with large repertoires are less likely to discriminate between neighbours and strangers. Across 34 species, we

found no evidence that songbirds with large repertoires are constrained in their ability to distinguish between neighbours and strangers. We conclude that large song repertoires do not inhibit neighbour–stranger discrimination in Red-eyed Vireos specifically, or songbirds generally.

Introduction

The sexual signals of animals play a central role in mate attraction and resource defence (Bradbury & Vehrencamp, 2011). Many animals produce individually distinctive signals (reviewed in Tibbetts & Dale, 2007) that facilitate individual identification of conspecific animals (e.g. Müller & Manser, 2007). During signalling interactions, any time an animal identifies a signaller it must decide whether the signaller is threatening or nonthreatening and respond appropriately. In territorial animals, unfamiliar 'strangers' are often considered a greater threat than familiar 'neighbours' because strangers may be prospecting for a breeding territory whereas neighbours should be encountered routinely and already possess a territory (Getty, 1987). This phenomenon of decreased aggression towards neighbours is known as the 'dear enemy effect' (Fisher, 1954) and has been found in a wide variety of taxa including reptiles (Ibáñez et al., 2013), birds (Linhart et al., 2012), mammals (Monclús et al., 2014), insects (Langen, et al., 2000), fish (McGregor & Westby, 1992), amphibians (Bee & Gerhardt, 2002) and crustaceans (Booksmythe et al., 2010) (reviewed in Temeles, 1994).

Many animals rely on vocal signals for territorial signalling (Bradbury & Vehrencamp, 2011). Acoustic signals are often individually distinctive, usually based on fine structural differences in the vocalizations of conspecific individuals (e.g. Arnold & Wilkinson, 2011; Bee et al., 2001; Kennedy et al., 2009). A compelling body of evidence supports the idea that animals differentiate among individually distinctive vocalizations, including operant conditioning or habituation–discrimination studies (e.g. Trefry & Hik

2009; Weary & Krebs, 1992) and field-based studies of free-living animals (e.g. Price et al., 2014; Wilson & Mennill, 2010). This acoustic discrimination ability allows animals to identify conspecific individuals and respond appropriately.

Birds show extensive variation in song repertoire sizes, ranging from 1 to over 2000 song types (MacDougall-Shackleton, 1997). Several studies have suggested that birds with large song repertoires display weaker discrimination abilities than birds with small song repertoires (Falls & D'Agincourt, 1981; Godard, 1993a; Hoelzel, 1986; Kroodsma, 1976; McGregor & Avery, 1986). There are at least three reasons why large song repertoires may impose a constraint on individual recognition: (1) birds must learn more songs to facilitate discrimination; (2) each song type will be heard less frequently, creating less opportunity to learn each song; and (3) song sharing may be higher, making identity assignment more difficult (Stoddard, 1996). In contrast, other studies have found that some bird species capably discriminate between conspecific individuals despite their large repertoires (Botero et al., 2007; Hyman, 2005; Weary et al., 1992). Additionally, two decades ago, a comparative analysis suggested that there was no relationship between repertoire size and neighbour–stranger discrimination ability across 20 species of passerines of the suborder Passeri (Weary et al., 1992). It is clear that evidence exists both to support and contradict the hypothesis that large repertoires constrain individual discrimination, and more research is needed to fully understand the effect repertoire size has on individual discrimination.

Red-eyed Vireos (*Vireo olivaceus*) are songbirds that have large vocal repertoires, with repertoire size estimates ranging from a median of 28.5 song types (Borror, 1981)

to 51 song types (Godard, 1993a). Previous research suggested that Red-eyed Vireos are unable to discriminate between individual neighbours due to their large repertoire size (Godard, 1993a). The distinction between individual neighbours is more difficult than the distinction between a neighbour and a stranger, because neighbours belong to the same class of conspecific individuals (i.e. familiar) whereas neighbours and strangers belong to different classes of conspecific individuals (i.e. familiar and unfamiliar) (Stoddard, 1996). Red-eyed Vireos present an interesting animal in which to study neighbour–stranger discrimination because of this previous work that has called their discrimination ability into question.

In this study we had two goals. (1) Employing the classic neighbour–stranger discrimination paradigm, we used a playback experiment to test the ability of Red-eyed Vireos to discriminate between neighbours and strangers. To complement this, we quantified repertoire size and song sharing at our study site. (2) Although many studies have discussed the negative effects that a large repertoire may have on an individual’s capability to discriminate neighbours from strangers (Falls & D’Agincourt, 1981; Godard, 1993a; Kroodsma, 1976), only one study (Weary et al., 1992) has examined this across multiple species. We sought to update this study with a comparative analysis of neighbour–stranger discrimination literature across the songbirds (i.e. birds in the order Passeriformes, suborder Passeri), using a phylogenetically controlled analysis to ask whether songbirds with large repertoires are less likely to discriminate between neighbours and strangers.

Field Study

Methods

General field methods

We conducted a playback study with Red-eyed Vireos at the Queen's University Biological Station (44°34'N, 76°19'W) north of Kingston, Ontario, Canada. We conducted playback experiments from 24 May to 4 July 2015, a time when all Red-eyed Vireos at our site had established their breeding territories and when most subjects were incubating eggs or in the early stages of chick rearing. We studied 28 males occupying breeding territories in eight different woodlots at our study site (average \pm SE distance between woodlots: 593.5 \pm 92.9 m, $N = 8$). The birds were not banded, and instead we relied on location information and features of acoustic recordings to distinguish between different males (sex was identified by song, because only males sing in this species; Cimprich et al., 2000). We identified individuals by following birds on their breeding territory, paying careful attention to the movement patterns of each of our subjects, monitoring the song posts and perches they used and the parts of the forest they occupied. We verified the identities of individuals by comparing recordings of the songs they sang during playback trials to songs we collected in previous focal recordings collected during observation sessions. We based our analyses of repertoire size and song sharing on 21 males where we had recorded at least 250 songs from each bird. We based our analysis of playback responses on 21 males (14 of these males were the same males used for repertoire and song-sharing analyses) after excluding three males due to

uncertainty regarding identity (no shared songs were detected during playback trials when compared to previous recording sessions), two males due to neighbour interference during playback, and two additional males due to a lack of response to our playback stimuli.

All methods involving animals were approved by the University of Windsor Animal Care Committee (AUPP number 13-15).

Song collection and playback stimuli

To quantify repertoire size and song sharing, and to gather sounds for playback stimuli, we recorded spontaneous bouts of song from male Red-eyed Vireos. We collected recordings with an omni-directional microphone (model: Sennheiser ME62/K6, Sennheiser, Wedemark, Germany) mounted in a parabolic reflector (model: Telinga MK2, Telinga Microphones, Uppsala, Sweden) connected to a digital solid-state recorder (model: Marantz PMD660, 44.1 kHz sampling rate, 16-bit encoding, Wave format). Before recording a bird, we followed it around its breeding territory for at least 30 min, paying careful attention to the bird's song posts and the locations of its neighbours, in order to be certain that we were recording the correct bird. We then collected at least 10 min of continuous song from each Red-eyed Vireo. After song collection, we hung flagging tape to mark the territory boundaries we had observed during the observation session.

We created playback stimuli using Audition 3.0 software (Adobe, San Jose, CA, U.S.A.). Playback stimuli were composed of 1 min of continuous Red-eyed Vireo song

repeated three times for a total of 3 min. From our field recordings, we selected the 1 min portion with the lowest level of background noise, based on visual assessment of sound spectrograms. We applied a 1000 Hz high-pass filter to filter out low-frequency background noise, below the range of Red-eyed Vireo songs. We then trimmed or added small sections of silence between songs, so that all songs were separated by intersong intervals of 0.6–0.8 s. Our preliminary analyses demonstrated that this was a natural song rate in our population. We normalized playback stimuli to -1 dB in Audition. In the field, we standardized the sound output from the loudspeakers using a sound level meter (Casella CEL-240; C-weighting, fast response) so that the peak amplitude of each stimulus was 80 dB at a distance of 1 m from the loudspeaker, a natural song amplitude for this species.

For each subject, 'neighbour stimuli' were songs recorded from a male that occupied a territory adjacent to the focal bird, and 'stranger stimuli' were songs recorded from birds that occupied a territory at least 1.5 km away from the focal bird. We chose this distance because Red-eyed Vireos do not move far from their territories after establishment, and therefore it is unlikely that a bird would hear the song of another conspecific 1.5 km away. In total, we created 32 stimuli for our 42 trials; 10 stimuli were used twice (five were used twice as strangers, four were used twice as neighbours and one was used once as a neighbour and once as a stranger).

Playback experiment

We carried out playback experiments between 0700 and 1100 hours, a time

when song rate is high for Red-eyed Vireos at our study site (C. Moser-Purdy, personal observation). Trials consisted of a 3 min playback period followed by a 10 min postplayback observation period. We flipped a coin before the first playback trial, to determine whether neighbour or stranger stimuli would be presented first; for all subsequent trials we alternated which stimulus was presented first. Across all 28 subjects, we played both neighbour and stranger playback first an equal number of times; however, out of the 21 subjects included in our final analysis, eight received neighbour playback first and 13 received stranger playback first. Neighbour and stranger playback trials took place on consecutive days except for three trials that took place 2 days apart and one trial that took place 3 days apart due to inclement weather.

We placed the loudspeaker (model: Scorpion TX200, FOXPRO, Inc., Lewistown, PA, U.S.A.) in a tree between 1.5 m and 2 m above the ground, roughly 10 m into the focal bird's territory, nearest to the boundary with its neighbour (as in Godard, 1993a). We set up flagging tape at 2 m and 5 m in four equally spaced directions from the loudspeaker to facilitate estimates of the distance of the focal bird from the loudspeaker. We began playback trials when the focal bird was singing at least 15 m away from the loudspeaker and the neighbour used to create the neighbour stimulus was silent. We placed the loudspeaker in the same location within the focal bird's territory for each playback trial. An observer (C.M.-P.) dictated the behaviour of the focal male and recorded the vocalizations of the focal male using a shotgun microphone (Sennheiser ME67/K6) connected to a solid-state digital recorder (Marantz PMD660, New York, NY, U.S.A.). The same observer (C.M.-P.) then scanned through the recordings

of the trial in Syrinx-PC (John Burt, Seattle, WA, U.S.A.) and annotated the spoken commentary of the bird's activities and the songs of the subject to create a time-stamped record of the bird's behaviour.

From the time-stamped record of the subjects' behaviour, we extracted the following response measures: distance of closest approach to the loudspeaker (in metres), latency to approach within 5 m of the loudspeaker (in seconds), duration of time spent within 5 m of the loudspeaker during the playback trial (in seconds), duration of time spent within 5 m of the loudspeaker in the post-playback observation period (in seconds), number of songs sung and number of soft songs sung. Soft songs were of unusually low amplitude and fairly easy to identify in the field after spending time observing Red-eyed Vireos throughout the 2014–2015 field seasons. Soft songs have received recent attention because they may be associated with aggressive intent in several songbird species (Akçay et al., 2015). There are no previous reports of soft song in Red-eyed Vireos; however, our preliminary observations suggested that they occur in this species, just as they appear to be common among many other songbirds (Dabelsteen et al., 1998; Reichard & Welklin, 2015).

Repertoire size and song sharing

To quantify repertoire size, we used Syrinx-PC to visualize spectrograms of the recordings we collected of spontaneously singing males. We calculated repertoire size for all birds where we had recordings of 250 or more songs ($N=21$). In most cases, we were able to collect all required songs during a single recording session. In cases where

this was not possible, we combined song types recorded on multiple days and compared song types between these days to ensure that the same bird was recorded. If the majority of song types were similar between the two recording sessions, we assumed that it was the same individual singing. Following Borror (1981), we considered syllables to be part of the same song when they were separated by less than 0.3 s of silence. Borror (1981) studied Red-eyed Vireo songs across the species' range in the United States and found that Red-eyed Vireos sing with immediate variety and that their song types are highly stereotyped across renditions. The songs in our recordings matched this pattern, and we found that new song types were simple to detect because songs were either notably different from each previous song type, or a perfect match with a previous song type. We developed a library of song types for each individual. For every song encountered in our recordings, we visualized it as a sound spectrogram in Syrinx-PC and compared it to all of the previous songs sung by that bird. If there was no match with a song in that bird's accumulated library, we added the new song to the library. We calculated repertoire size as the total number of song types sung over the recordings we had for each bird. To determine whether our recordings were adequate to estimate the full repertoire size of each bird, we plotted the number of new songs sung over the total number of songs sung (Fig. 1). If this graph approached a horizontal asymptote, we assumed that we had recorded a complete or near-complete recording of the bird's full repertoire (as in Godard, 1993a).

Using the song libraries we developed for each of the 21 males, we measured song sharing between Red-eyed Vireos. For this analysis, we focused on 10 neighbouring

pairs and 7 non-neighbouring pairs. We defined non-neighbours as birds that occupied the same woodlot but did not share a territory boundary. We compared a bird's repertoire to all possible neighbours and non-neighbours for which we had recordings. We compared each song from each male's song library to the songs in the second male's song library, and assigned each song a status of 'shared song' or 'unshared song'. We considered a song to be shared when songs had nearly identical fine structural features including bandwidth, length and shape features (see Fig. 2.1). We calculated the degree of pairwise song sharing using the standard song-sharing index: $2 \times (\text{number of songs shared between two individuals}) / (\text{repertoire size of individual 1} + \text{repertoire size of individual 2})$ (Harris & Lemon, 1972).

Statistical analysis

We conducted statistical analyses using R (v.3.2.3, R Development Core Team, 2015). Our song-sharing data and playback response data both showed a non-normal distribution (Shapiro–Wilk tests: song sharing: $W = 0.86$, $N = 20$, $P = 0.008$; closest approach to the loudspeaker: $W = 0.78$, $P < 0.0001$; latency to approach within 5 m of the loudspeaker: $W = 0.70$, $P < 0.0001$; time spent within 5 m of the loudspeaker during playback: $W = 0.84$, $P < 0.0001$; time spent within 5 m of the loudspeaker after playback: $W = 0.80$, $P < 0.0001$; number of songs sung: $W = 0.94$, $P = 0.02$; number of soft songs sung: $W = 0.56$, $P < 0.0001$; all $N = 21$) likely due to a preponderance of minimum and maximum values. For our playback response data, we used an exact Wilcoxon signed-ranks test using the package `exactRankTests` (Hothorn & Hornik, 2015). To correct for

multiple comparisons given the six response variables analysed, we applied a Bonferroni correction. Tests were considered significant if they had a *P* value of less than 0.008. To determine difference in song sharing between neighbours and non-neighbours occupying the same woodlots, we used an exact Wilcoxon two-sample test using the package `exactRankTests`.

Results

Playback experiment

Red-eyed Vireos showed a more intense response to strangers than to neighbours for four of six response variables: closest approach to the loudspeaker, time spent within 5 m of the loudspeaker during playback, latency to approach the loudspeaker and number of soft songs sung (Fig. 2.2a–c, f, Table 2.1). Red-eyed Vireos showed an equal response to neighbours and strangers for the remaining two variables: total number of songs sung over the entire trial and the time spent within 5 m of the loudspeaker during the postplayback observation period (Fig. 2.2d, e, Table 2.1).

Repertoire size and song sharing

Red-eyed Vireos in our study population had large song repertoires (median \pm inter-quartile range male repertoire size = 44 ± 16 song types, range 23–91; Fig. 2.3), consistent with three previous studies of this species (Borror, 1981; Godard, 1993a; Lemon, 1971). For nine birds where repertoire size approached an obvious horizontal

asymptote, median repertoire size was 34 ± 1.5 song types (range 23–55).

Red-eyed Vireos showed very low levels of song sharing. The median \pm inter-quartile range song-sharing index was $6.5 \pm 2.2\%$ (range 2.5–7.8%) for neighbours and $2.2 \pm 0.4\%$ (range 0–2.8%) for non-neighbours. Song sharing was significantly higher between neighbours than between non-neighbours (exact Wilcoxon two-sample test: $W = 3$, $N = 17$, $P = 0.0004$).

Comparative Analysis

Methods

Data collection

To better understand the effect of repertoire size on neighbour–stranger discrimination at a broader scale, we conducted a comparative analysis across the published literature on songbirds. We compiled repertoire sizes for all neighbour–stranger discrimination studies, to our knowledge, that have been conducted on male songbirds (i.e. birds in the suborder Passeri within the order Passeriformes) using song playback and without other experimental manipulations (e.g. experimentally varying plumage colour in Blue Tits (*Cyanistes caeruleus*; Poesel et al., 2007). We excluded studies that used altered song types of neighbours to imitate strangers (e.g. Aubin et al., 2004; Osiejuk, 2014) because these experiments do not directly test neighbour–stranger discrimination but rather what elements of a song are used for individual discrimination. We chose to focus on songbirds because they have received extensive study, because

song repertoires are common (MacDougall-Shackleton, 1997), and because there have been numerous experiments on neighbour–stranger discrimination in these animals (Stoddard, 1996). We compiled repertoire sizes preferably based on empirical studies. When this was not possible, we used rough estimates of repertoire size found in the literature (preferably the same paper with the neighbour–stranger discrimination experiment). Note: Skylark, *Alauda arvensis*, repertoire size was given as syllable repertoire size since Skylarks sing long continuous songs and syllable repertoire is more indicative of the complexity of their repertoire (Briefer et al., 2008), and Stripe-backed Wren, *Campylorhynchus nuchalis*, repertoire size was given as the duet repertoire size because duets were used as the playback stimuli in this study (Wiley & Wiley, 1977).

We were interested in investigating whether the strength of neighbour–stranger discrimination could be influenced by repertoire size. We calculated effect sizes of responses between neighbours and strangers as our metric for strength using Cohen’s d . Our analysis focuses on 34 species where the playback study reported information that allowed us to calculate effect size, Cohen’s d , for the strength of the difference in response to strangers versus neighbours. We calculated Cohen’s d using means and standard errors or deviations as reported in the original studies, but when these were not provided, we used the test statistics given in the original studies. We used the response measure that gave the highest Cohen’s d for our analysis. For articles that did not present their means and standard deviations or errors in text form, but presented these values in graphs, we manually measured the graphs using a ruler with the graph zoomed to fill the screen.

Statistical analysis

We used a phylogenetic generalized least squares analysis (PGLS) to analyse our comparative data, to test for an effect of repertoire size on the effect size of the difference in response to neighbours versus strangers, while controlling for phylogeny. We downloaded 1000 phylogenetic trees using the Hackett sequence-based data set (Jetz et al., 2012; www.birdtree.org). Using TreeAnnotator (v.1.8.2; Drummond et al., 2012), we calculated a maximum clade credibility tree with burn-in value set to 0, posterior probability limit set to 0, and node heights as median heights. We used the package ‘caper’ (Orme, 2013) implemented in R. Our data overlapped with 17 of the 20 studies that Weary et al. (1992) used in their comparative analysis (we excluded two studies due to lack of sufficient information for calculating Cohen’s d , and a third study because we did not agree that it tested neighbour–stranger discrimination; Godard, 1991); our data add 17 more species to this comparative analysis.

Results

We found 38 species of songbirds that have been the subject of an investigation of neighbour–stranger discrimination studies, representing a broad spectrum of families of songbirds. Across these 38 species, 33 showed discrimination between neighbours and strangers and five did not (Table 2.2). For 34 of these species, we found information on species-typical repertoire sizes and sufficient data to calculate effect size (Cohen’s d) of the strength of their response to neighbours versus strangers. We found no significant relationship between repertoire size and the strength of the response to strangers

versus neighbours (PGLS: $R^2 < 0.0001$, $N = 34$, $P > 0.99$; Fig. 2.4).

Discussion

Red-eyed Vireos readily discriminated between neighbours and strangers in four out of six variables. For each of these four variables, Red-eyed Vireos responded more intensely to stranger playback than to neighbour playback, thus exhibiting the dear enemy effect. Red-eyed Vireos at our study site had large repertoires and displayed low levels of song sharing, with greater song sharing between neighbouring birds than between non-neighbouring birds occupying the same woodlot. We found no effect of repertoire size on the strength of discrimination in our comparative analysis. Our evidence shows that large repertoires do not inhibit neighbour–stranger discrimination in Red-eyed Vireos or songbirds as a whole.

Neighbour–Stranger Discrimination

Our results indicate that Red-eyed Vireos are capable of acoustically differentiating between neighbours and strangers, even though they have large vocal repertoires. Red-eyed Vireos approached closer to the loudspeaker, spent more time within 5 m of the loudspeaker during playback, had a lower latency to approach the loudspeaker within 5 m and sang more soft songs during stranger trials than during neighbour trials. These physical response variables are all indicative of an aggressive response to an intruding individual. An increased number of soft songs is also indicative

of an aggressive response, in line with previous studies that have found similar results (e.g. Akçay et al., 2011). Soft songs have received substantial attention recently, but little is known about why birds use them to indicate aggressive intent and how these signals remain reliable (Akçay et al., 2015). Despite this, a wide array of animals, including birds (Akçay et al., 2015) and some mammals (Gustison & Townsend, 2015), are known to use soft vocalizations in aggressive contexts, and Red-eyed Vireos now join these animals.

Two measures of Red-eyed Vireos' playback responses showed no differences in response to neighbours versus strangers. During the postplayback observation period, there was no significant effect of treatment on the amount of time spent 5 m from the loudspeaker. Shortly after the playback ended, we often observed subjects vacate the area near the loudspeaker and sing from various locations in their territory, which may explain the lack of significance in this result. Red-eyed Vireos did not differ in the number of songs they sang during neighbour and stranger playback trials. Red-eyed Vireos have a high singing rate (Borror, 1981), so this result is likely due to Red-eyed Vireos spontaneously singing their territorial songs during neighbour playback trials rather than singing their territorial songs as an aggressive response to the playback. Indeed, this is what we observed in the field. Furthermore, outside of soft songs, there is little evidence in other bird species that singing behaviour is a signal of aggressive intent and physical responses may be more reliable indicators of aggression than vocal behaviours (Searcy & Beecher, 2009; Searcy et al., 2006).

Our results suggest that Red-eyed Vireos perceive strangers as a greater threat

than neighbours during the breeding season. Since neighbours represent a lesser threat than strangers (Temeles, 1994), territorial individuals benefit from expending energy on tasks such as foraging when hearing neighbour song instead of engaging in territory defence, a behaviour more appropriate when hearing nearby stranger song. The dear enemy effect, and more broadly neighbour–stranger discrimination, appears to be a common phenomenon across a wide array of territorial taxa (Temeles, 1994). In territorial animals, it may be wise to assume neighbour–stranger discrimination is present *a priori* due to the taxonomic breadth of this phenomenon.

A previous study by Godard (1993a) found no difference between the responses of Red-eyed Vireos to neighbour playback near their shared boundary and neighbour playback near an unshared boundary. It is possible that Godard’s result was due to the inability of Red-eyed Vireos to differentiate between the more difficult distinction of two familiar individuals (i.e. two neighbours) compared to our study where we investigated the ability of Red-eyed Vireos to make the simpler distinction between a familiar and unfamiliar individual (i.e. a neighbour versus a stranger). However, due to the low amount of song sharing between neighbours, and thus the high amount of individually unique songs each Red-eyed Vireo sings, it seems unlikely that Red-eyed Vireos would be incapable of differentiating between individual neighbours. One alternative explanation for these results may be that Red-eyed Vireos perceive neighbours as an equal threat regardless of location, warranting an equal response to playback of all neighbour stimuli. Godard (1993a) did not give the breeding stage of her study subjects, and her playback may have been conducted during the fertile period when neighbours

may represent an increased threat due to extrapair paternity. Red-eyed Vireos have high levels of extrapair paternity (58% of nestlings; 57% of broods; Morton et al. 1998), and neighbours are the most common sires of extrapair offspring (Griffith et al., 2004; Hill et al., 2011; Mennill et al., 2004), suggesting that neighbours may be an increased threat during fertile periods. We conducted our study during the incubation and chick-rearing stages, when neighbours were no longer a threat to extrapair paternity and territory borders were well established. Further research exploring seasonal variation in neighbour–stranger discrimination, across different stages of female fertility, is a worthy area of future study.

Future work examining the neighbour–neighbour discrimination ability of Red-eyed Vireos may benefit from using a design similar to that of Godard (1993b) or Akçay et al. (2009), wherein researchers simulated the intrusion of a neighbour and then observed whether the subjects responded more aggressively to this ‘uncooperative’ neighbour after the intrusion. This would provide a more rigorous test of Red-eyed Vireos’ abilities to discriminate between familiar conspecifics by increasing their motivation to respond more aggressively to an intruding bird.

Repertoire Size and Song Sharing

Red-eyed Vireos in our study population had large repertoires (median of 44 song types), in line with previous studies that have found similar results (median: 28.5 song types, $N=38$, range 12–73, Borror, 1981; median: 51 song types, $N=5$, range 31–95, Godard, 1993a). Our results should be interpreted as conservative estimates of

repertoire sizes, however, because many of our birds had not approached a repertoire asymptote (Fig. 1). Interestingly, repertoire size seems to be highly variable in this species, with a wide range of 23–91 song types in this study, 12–117 song types found by Borror (1981) and 31–95 song types found by Godard (1993a). Large repertoires may be indicative of male quality (Catchpole & Slater, 2008). Evidence for this hypothesis exists in several bird species where larger song repertoires are correlated with male fitness indicators such as body size, territory tenure and reproductive success (e.g. Hiebert et al., 1989; Kipper et al., 2006; Hesler et al., 2012; Reid et al. 2005; but see Beecher et al., 2000). Because of the large individual variation in Red-eyed Vireo song repertoires, it may represent an ideal species in which to further test this hypothesis.

Red-eyed Vireos appear to have considerably low song sharing between individuals, with slightly higher sharing with neighbours than with non-neighbours from the same site. This pattern is commonly found across many bird species (e.g. Foote & Barber, 2007; Griessmann & Naguib, 2002; Mennill & Vehrencamp, 2005; Price & Yuan, 2011), although in many of these species, song sharing is considerably higher than what we report in Red-eyed Vireos. However, despite the low song sharing in Red-eyed Vireos, they still follow this pattern of higher song sharing between neighbours than between non-neighbours (but see Borror, 1981). Because of the low song sharing that we found, it is likely that Red-eyed Vireos can distinguish among different individuals based on songs that are unique to each individual, with what are sometimes called ‘signature songs’ (Weary et al., 1990). Since each Red-eyed Vireo in this study sang many songs that their neighbours did not sing, individuals of this species should not have

difficulty differentiating between conspecific individuals. Note, however, that in most cases, we did not record repertoires of each individual's neighbours to determine song sharing between all neighbours and likely did not record full repertoires for all individuals; thus some shared songs may have been missed (see Fig. 1). Still, given the low song sharing observed in this study, even for the animals whose repertoires reached an asymptote, it is unlikely that the individuals in this large-repertoire species would share all of their songs with their neighbours. O'Lochlen and Beecher (1999) found that female Song Sparrows are capable of discriminating between males based on unshared song types, and there is evidence that Great Tits (*Parus major*) may discriminate more strongly based upon unshared songs of neighbours than on shared songs of neighbours (McGregor & Avery, 1986). Alternatively, Red-eyed Vireos may discriminate between individuals based on individually distinctive vocal characteristics; Weary and Krebs (1992) found that great tits were capable of classifying unheard songs to the correct bird after being trained with other songs from the same bird's repertoire, suggesting that their songs have unique vocal characteristics. Red-eyed Vireos may also discriminate between neighbours based on fine structural differences between shared vocalizations as found in many other animals (Digweed et al., 2012; Osiejuk, 2014). Our results suggest that Red-eyed Vireos are capable of discriminating conspecific individuals based on songs, but more research is needed to elucidate song features that facilitate this discrimination.

Comparative Analysis

Repertoire size did not have a significant effect on a bird's ability to discriminate between neighbours and strangers. This is unsurprising as many large-repertoire birds such as the European Robin (*Erithacus rubecula*; Brindley, 1991) and now the Red-eyed Vireo, discriminate between neighbours and strangers, whereas some birds with small repertoires do not exhibit behavioural discrimination between neighbours and strangers, like the Chipping Sparrow (*Spizella passerina*; Albrecht & Oring 1995). A previous study examined the effect of repertoire size on the strength of response to neighbours and strangers and found no evidence to support the hypothesis that repertoire size constrains discrimination (Weary et al., 1992), and our updated analysis with 17 additional species confirms this position. The idea that repertoire size may constrain neighbour–stranger discrimination and conspecific discrimination has received little recent support despite early findings that supported this hypothesis (e.g. Falls & D'Agincourt, 1981; Kroodsma, 1976). Given the results of the current study and those of various previous studies on recognition abilities of large-repertoire birds (e.g. Briefer et al., 2008; Jaška et al., 2015; Ritchison, 1988), it is abundantly clear that large repertoires do not constrain conspecific discrimination abilities.

Large repertoires may be positively correlated with male quality, allowing females to select the best males based on repertoire size (Catchpole & Slater, 2008). Future studies may benefit from an examination of dominance and variation in male response to conspecific individuals with large and small repertoires. Some work has been done on this topic. For example, Yasukawa (1981) found that Red-winged

Blackbirds (*Agelaius phoeniceus*) were less likely to intrude on a territory when multiple song types were broadcast than when only one song type was broadcast. However, other studies have found that repertoire size had no effect on response to playback (Balsby & Dabelsteen, 2001; Hesler et al., 2011). Red-eyed Vireos may be an ideal species in which to study the effect of repertoire size on male–male interactions due to the wide range of repertoire sizes reported here and in other studies (Borror, 1981; Godard, 1993a).

Conclusions

Large repertoires do not constrain neighbour–stranger discrimination. The Red-eyed Vireo, a large-repertoire songbird, capably discriminated between neighbours and strangers. Furthermore, we found no relation between repertoire size and neighbour–stranger discrimination in a comparative analysis across 34 passerines of the suborder Passeri. Future studies involving repertoire sizes may benefit from a focus on male response to varying repertoire sizes.

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Tables

Table 2.1. Red-eyed Vireos responded more strongly to strangers than to neighbours in four out of six response measures. We used an exact Wilcoxon signed ranks test to determine significance. Significant outcomes ($P < 0.008$) are shown in bold.

Response measure	Mean + SE		<i>W</i>	<i>P</i>
	Neighbour	Stranger		
Closest approach to loudspeaker (m)	7.9±1.6	1.6±0.5	179.5	0.0002
Latency to approach within 5 m of loudspeaker (s)	462.9±71.7	95.6±35.2	204	0.00003
Time (s) within 5 m of loudspeaker during playback	26.9±10.6	87.8±8.6	12	0.0001
Time (s) within 5 m of loudspeaker after playback	86.0±25.4	157.5±36.4	48	0.06
Total number of songs sung	225.3±39.9	270±46.4	84	0.29
Total number of soft songs sung	2.1±1.4	19.4±6.2	9.5	0.002

Table 2.2. Comparative analysis of neighbour–stranger discrimination in 38 songbird species

Species	Repertoire size	Source	
		Neighbour–stranger discrimination	Repertoire size
Neighbour–stranger discrimination			
<i>Agelaius phoeniceus</i>	4.3	Yasukawa et al. (1982)	Yasukawa (1981)
<i>Alauda arvensis</i>	70.7	Briefer et al. (2008)	Briefer et al. (2011)
<i>Baeolophus bicolor</i> ¹	12.3	Schroeder & Wiley (1983)	Dugay & Ritchison (1998)
<i>Campylorhynchus nuchalis</i>	5	Wiley & Wiley (1977)	Wiley & Wiley (1977)
<i>Catharus fuscescens</i>	3	Weary et al. (1987)	Weary et al. (1987)
<i>Cettia diphone</i>	4	Momose (2000)	Hamao & Ueda (2000)
<i>Emberiza citrinella</i>	2.2	Hansen (1984)	Caro et al. (2009)
<i>Emberiza elegans</i>	59	Hwang & Park (1996)	Zeng et al. (2007)
<i>Emberiza fucata</i>	2	Hwang & Park (1996)	Kim & Park (1993)
<i>Emberiza hortulana</i>	4.2	Skierczynski et al. (2007)	Osiejuk et al. (2003)
<i>Erithacus rubecula</i>	175	Brindley (1991)	Brindley (1991)
<i>Fringilla coelebs</i>	2.9	Pickstock & Krebs (1980)	Slater (1981)
<i>Geothlypis trichas</i>	1	Wunderle (1978)	Wunderle (1978)
<i>Icteria virens</i> ²	62.2	Ritchison (1988)	Dussourd & Ritchison (2003)
<i>Liocichla steerii</i> ²	Unknown	Weng et al. (2012)	
<i>Melospiza georgiana</i>	3.5	Searcy et al. (1981)	Searcy et al. (1981)
<i>Melospiza melodia</i>	9.6	Harris & Lemon (1976); Kroodsma (1976); Stoddard et al. (1990)	Wilson et al. (2000)
<i>Parus major</i>	3	Järvi et al. (1977); Krebs (1971)	McGregor et al. (1981)
<i>Parus venustulus</i>	5	Wei et al. (2011)	Wei et al. (2011)
<i>Passerina cyanea</i>	1	Belcher & Thompson 1969; Emlen (1971)	Payne (1982)
<i>Phoenicurus ochruros</i>	2.4	Draganoiu et al. (2014)	Draganoiu et al. (2014)
<i>Seiurus aurocapilla</i>	1	Weeden & Falls (1959)	Lein (1981)
<i>Setophaga petechia</i>	11.8	Weary et al. (1992)	Beebee (2002)
<i>Setophaga ruticilla</i>	4.4	Weary et al. (1992)	Lemon et al. (1985)
<i>Spizella pusilla</i> ²	2	Goldman (1973)	Nelson & Croner (1991)
<i>Sturnella neglecta</i>	7.1	Falls & D'Agincourt (1981)	Falls & D'Agincourt (1981)
<i>Thryophilus pleurostictus</i>	19.7	Molles & Vehrencamp (2001)	Molles & Vehrencamp (1999)
<i>Thryothorus ludovicianus</i>	32.4	Hyman (2005); Shy & Morton (1986)	Morton (1987)
<i>Troglodytes troglodytes</i> ¹	6	Courvoisier et al. (2014)	Camacho-Schlenker et al. (2011)
<i>Vermivora celata</i>	1	Yoon et al. (2012)	Yoon et al. (2012)
<i>Vireo olivaceus</i>	44	Present study	Present study
<i>Zonotrichia albicollis</i> ²	1	Brooks & Falls (1975a, 1975b)	Wasserman (1979)
<i>Zonotrichia leucophrys</i>	1	Baker et al. (1981); Falls (1969)	Nelson & Poesel (2010)
No neighbour–stranger discrimination			
<i>Mimus gilvus</i> ³	130	Botero et al. (2007)	Botero et al. (2007)
<i>Phylloscopus trochilloides</i>	8.5	Katti (2001)	Irwin (2000)
<i>Spizella passerina</i>	1	Albrecht & Oring (1995)	Albrecht & Oring (1995)
<i>Sturnella magna</i>	61.3	Falls & D'Agincourt (1981)	Falls & D'Agincourt (1981)
<i>Thryophilus rufalbus</i>	10.8	Battiston et al. (2015)	Mennill & Vehrencamp (2005)

¹ Shows stronger response to neighbours than to strangers.

² Excluded from our calculation of effect size of the intensity of the difference in response to neighbours versus strangers because of insufficient data.

³ Shows recognition of other classes of conspecific individuals.

Figures

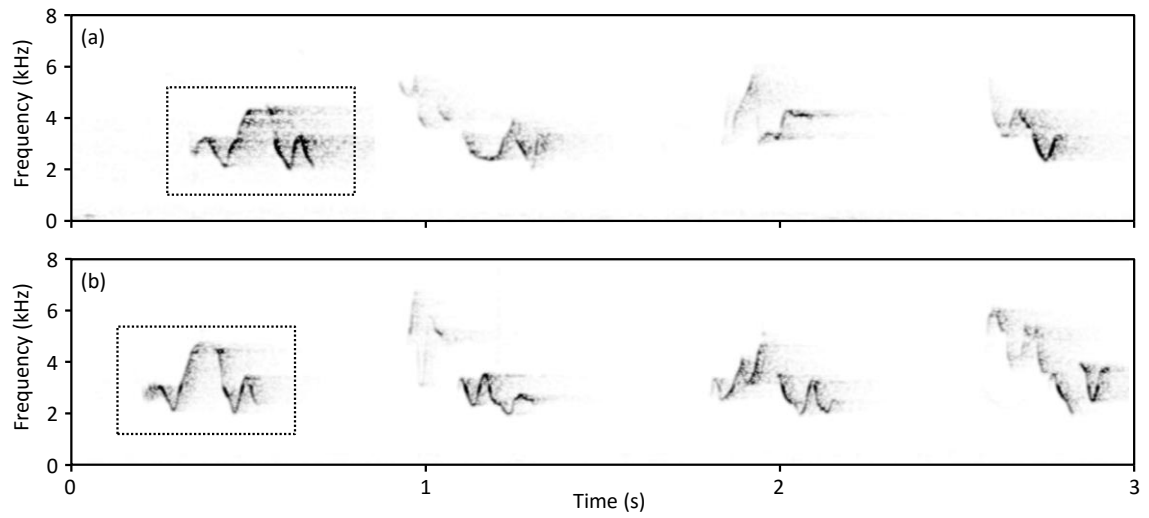


Figure 2.1. Two spectrograms from neighbouring Red-eyed Vireos. Boxes denote a shared song type.

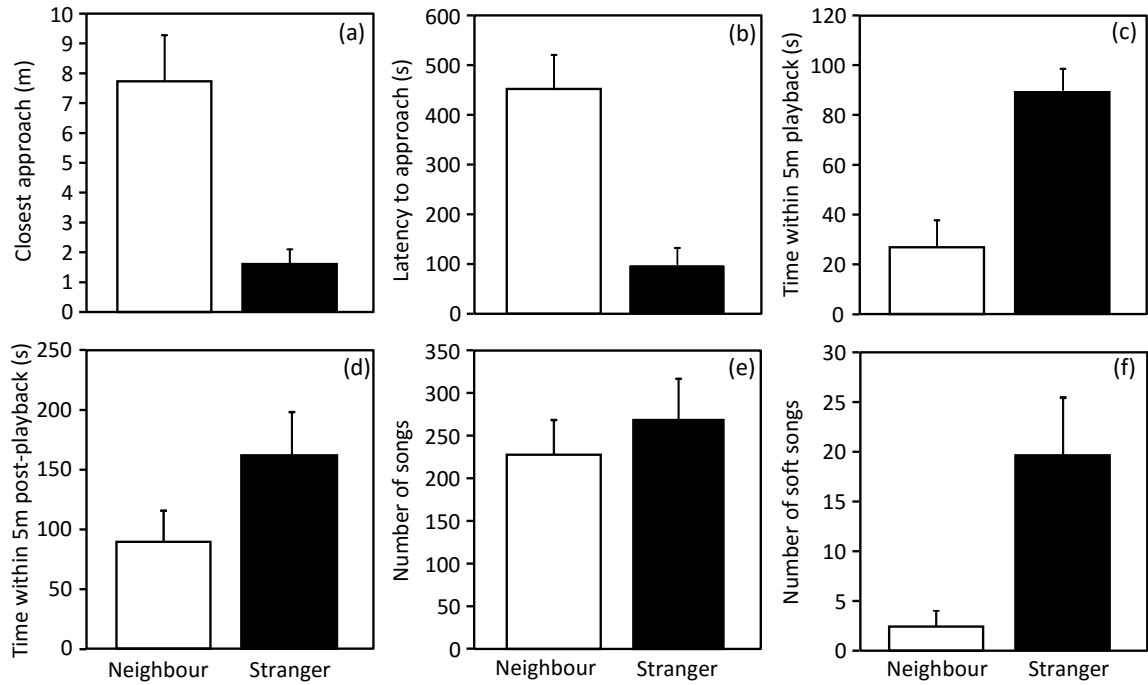


Figure 2.2. Red-eyed Vireos' responses to stranger and neighbour stimuli for each of the six variables examined.

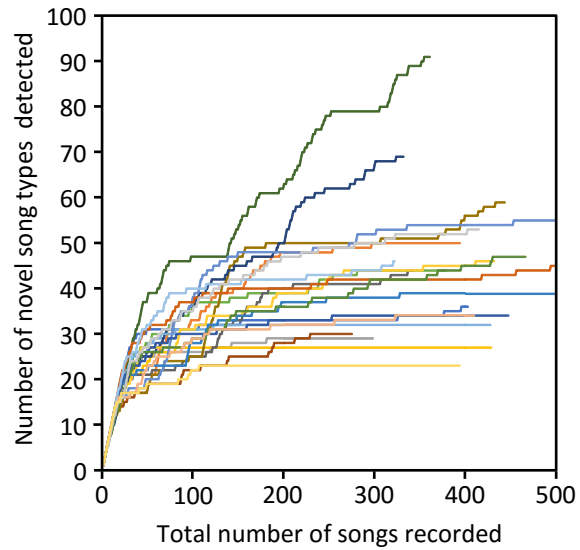


Figure 2.3. Repertoire size accumulation curves for 21 Red-eyed Vireos, each coded by a different colour. When a curve approaches an asymptote, such as the lowest curve in the figure, repertoire sampling can be assumed to be complete, given that no new song types are sung as the bird continues to cycle through its repertoire. In our data set, repertoire sampling was complete for nine individuals and incomplete for 12 individuals.

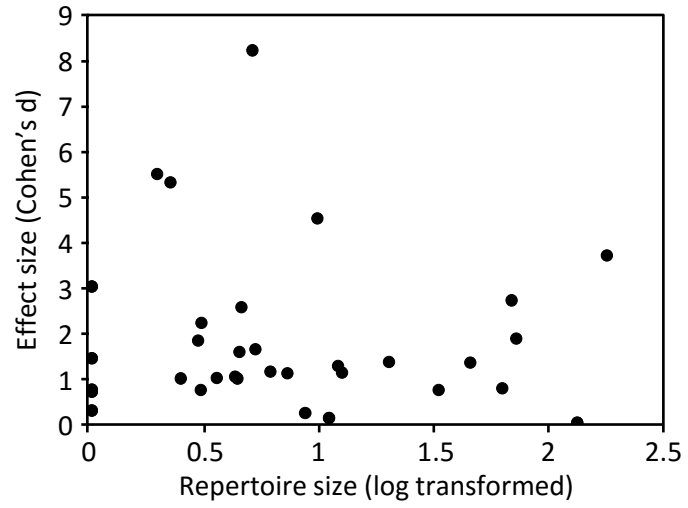


Figure 2.4. Results of a comparative analysis of 34 songbird species' log repertoire sizes with their effect sizes in response to neighbours and strangers ($R^2 < 0.0001$, $P > 0.99$).

CHAPTER 3

ENEMIES AREN'T ALWAYS DEAR: MALE SONG SPARROWS ADJUST EXPRESSION OF THE DEAR ENEMY EFFECT IN RESPONSE TO FEMALE FERTILITY

Chapter Summary

The dear enemy effect arises when territorial animals respond more intensely to unfamiliar strangers than to familiar neighbours. This widespread behavioural phenomenon occurs because strangers represent a threat to both an animal's territory and parentage, whereas neighbours represent a threat only to parentage. Recent research in birds demonstrates some flexibility in the dear enemy effect across the breeding season. Given that neighbours often sire extra-pair young, male animals may benefit by responding more aggressively to neighbours during periods of female fertility. Here we investigate the hypothesis that the dear enemy effect varies with female fertility by testing the prediction that birds will respond more strongly to neighbours when females are fertile than when they are not fertile. We conducted a playback experiment with wild Song Sparrows (*Melospiza melodia*), repeating playback sessions to paired territorial males over the course of a breeding season, including periods when females were fertile and periods when they were not. Male Song Sparrows displayed a dear enemy effect when their social mate was not fertile but did not display a dear enemy effect when their social mate was fertile. We conclude that Song Sparrows adjust behaviour towards neighbours based on their own mate's fertility status. We argue that this variation occurs because neighbours threaten a territorial male's parentage during his breeding partner's fertile period. These results suggest that male Song Sparrows increase aggression toward neighbours as a mate-guarding tactic.

Introduction

When an animal encounters a conspecific rival, it must choose an appropriate response. If the rival represents a high threat to the animal's resources or reproductive success, then the animal may respond aggressively; if the rival represents a low threat, then the animal may respond less aggressively or not at all. In territorial animals, unfamiliar conspecific rivals usually represent a greater threat because they may usurp an animal's territory or threaten an animal's paternity by copulating with its mate. Neighbours, in contrast, already occupy a territory of their own and therefore only threaten an animal's paternity (Temeles, 1994). Therefore, territorial male animals often respond more aggressively to unfamiliar stranger individuals than familiar neighbour individuals. This phenomenon is known as the "dear enemy effect" (Fisher, 1954). Decreased aggression toward neighbours allows animals to spend more time on important tasks such as foraging, nest building, or caring for young instead of engaging in costly territorial disputes. The dear enemy effect has been documented in diverse animal taxa, including insects (e.g. Langen et al., 2000), birds (e.g. Hardouin et al., 2006), mammals (e.g. Monclús et al., 2014), reptiles (e.g. Whiting, 1999), crustaceans (e.g. Booksmythe et al., 2010), fish (e.g. McGregor & Westby 1992), and amphibians (e.g. Feng et al., 2009).

Recent research on neighbour-stranger discrimination has revealed that the level of aggression displayed toward conspecific neighbours varies across the breeding season. Male Skylarks (*Alauda arvensis*) responded more strongly to strangers than

neighbours in the middle of a breeding season (i.e. after hatching of first brood) but displayed no difference in response during the beginning (i.e. territory establishment) or end of the breeding season (i.e. after hatching of second brood; Briefer et al., 2008). Additionally, Winter Wrens (*T. troglodytes*) increased their responses to neighbours versus strangers at the beginning of the breeding season but displayed no difference in response during the middle or end of the breeding season (Courvoisier et al., 2014). According to the Threat-level Hypothesis (Temeles, 1994), these results may be explained by a change in threat level during different stages of the breeding season. Although these studies have found differences in responses to neighbours and strangers across a breeding season, they did not directly investigate the underlying causes for these differences. Currently, the causes of the flexibility of the dear enemy effect across a breeding season are poorly understood.

In many bird species, neighbouring males are often the sires of extra-pair offspring, that is, offspring whose biological father is different from their social father (e.g. Hill et al., 2011; Gibbs et al., 1990; Griffith et al., 2002; Mennill et al., 2004). Therefore, during periods of female fertility, a neighbouring male should represent a greater threat to a male's paternity than at other times of the year. Consequently, a territorial male animal may benefit from responding aggressively to neighbouring males during his female's fertile period in order to protect his paternity. Conversely, neighbours should not be as threatening to a male during periods where his female is not fertile because they no longer threaten his paternity (see Fig. 3.1). Neighbours are expected to benefit from decreased aggression toward one another during these

periods so they can focus on foraging or provisioning young. Strangers, in contrast, should represent an equivalent threat across a breeding season because the loss of a breeding territory will always result in reduced reproductive success. We hypothesize that male expression of the dear enemy effect should vary with female fertility: the dear enemy effect should be present when females are not fertile but should be absent when females are fertile.

In this study, we tested this hypothesis by conducting repeated playback of neighbour and stranger songs during different breeding stages in Song Sparrows (*Melospiza melodia*). Song Sparrows are temperate-breeding songbirds that are known to display the dear enemy effect (Harris & Lemon, 1972; Kroodsma, 1976; Stoddard et al., 1990; Stoddard et al., 1991). This species has moderately high rates of extra-pair fertilization (e.g. 24.0% of chicks, 36.1% of broods, Hill et al. 2011; 10.5% of chicks, 20.0-40.0% of broods, Major & Barber 2004; 27.9% of chicks, O'Connor et al., 2006; 28.0% of chicks, 44.0% of broods, Sardell et al., 2010), and neighbours are the typical extra-pair sires (Hill et al., 2011). We predicted that if dear enemy effect expression is influenced by female fertility, then male Song Sparrows would respond more intensely (e.g. more flights, more time spent near the loudspeaker) to strangers than neighbours during periods when females were not fertile, but respond similarly to neighbours and strangers during periods when females were fertile. However, if dear enemy effect expression is not driven by female fertility status, we expected that male Song Sparrows would not differ in their aggression toward neighbours in a way that varies with female fertility.

Methods

Study site and study species

We conducted this experiment at the Queen's University Biological Station (44° 34' N, 76° 19' W) north of Kingston, Ontario, Canada. Our playback experiments took place between April 18 and May 22, 2015 and between April 8 and May 15, 2016; these periods correspond roughly to pair formation through nest-building, laying, and incubation in our study population. Our subjects were 29 focal male Song Sparrows (19 in 2015 and 10 in 2016) living in fields and marshes in the vicinity of the research station. Of our 29 subjects, 25 were banded with unique combinations of coloured leg bands and a Canadian Wildlife Services numbered band to facilitate individual identification. For the remaining four unbanded males, we distinguished between individuals based on recordings of their individually-distinctive song types, as well as their territorial position relative to landmarks and other nearby animals. Sharing of complete song types between neighbours is rare for Song Sparrows in eastern North America (Hughes et al., 2007; although see Foote & Barber 2007), including in our study population (Stewart & MacDougall-Shackleton, 2008) and therefore distinguishing between individuals based on unique song types is not difficult. From the original 29 playback subjects, we excluded two individuals that did not respond to playback, three individuals that never paired with a female, and two individuals that moved their breeding territory part way through the study period. After these exclusions we were left with 22 males for our analyses.

Playback Stimuli

We created playback stimuli that allowed us to simulate song bouts of neighbours and strangers for each of our playback subjects. We considered neighbours to be birds that occupied a territory adjacent to the playback subject (i.e. some portion of their territory boundary was shared) and we considered strangers to be birds that occupied a territory at a different site, at least 2 km away from the focal bird. Male Song Sparrows in this population usually move less than 200 m between breeding attempts (Potvin et al., 2015) so it is very unlikely that focal males would have previously encountered these stranger stimuli.

To create playback stimuli, we collected recordings of Song Sparrows between 0600h and 1200h during early and mid-April using a directional microphone (Sennheiser ME67/K6) connected to a solid-state digital recorder (Marantz PMD660, 44.1 kHz sampling rate, 16-bit encoding, WAVE format). Birds were usually recorded singing spontaneous territorial songs, however in a few instances we used a short playback (<30 s) to motivate birds to sing. From each recording of each male, we extracted five songs, each of a different song type, choosing the recordings with the lowest level of background noise (based on visual inspection of sound spectrograms generated in Audition 3.0 software, Adobe, San Jose, CA). For each song we applied an 800-Hz high-pass filter to remove low-frequency background noise. In a few recordings, higher-frequency noise was present; we reduced it to background levels using the lasso tool and the amplify function of Audition. After filtering sounds, we normalized all playback stimuli to -1 dB; we standardized the amplitude of our loudspeaker (model: Scorpion

TX200, FOXPRO Inc.) so that the peak amplitude of each stimulus was 75 dB at a distance of 1.0 m from the speaker (sound level meter: Casella Cel-240; C weighting, fast response). We then repeated each song at a rate of 1 song every 10 seconds (a natural rate for Song Sparrows based on preliminary observations), for a total stimulus track duration of 3 mins.

For every bird that we simulated with playback, we created five different stimuli, each with a different song type from the same recorded male (although in two cases we were only able to collect four well-recorded song types). Across repeated trials to each subject, we cycled through the five (or four in two cases) different stimuli for each of the simulated intruders. All focal birds received playback from the same neighbour bird and stranger bird during all trials. However, in three instances, the neighbour lost its territory to a different bird in the middle of the experimental period; in these cases, we changed the neighbour stimulus to simulate the current neighbour, thereby maintaining an experimental design of “neighbour versus stranger”.

Playback Experiment

We mapped the territories of our focal birds during their arrival from migration in early April. An observer (CM-P) followed focal birds around their territories for at least 90 min, taking careful note of locations where each bird sang, and logging these points into a GPS (Garmin GPS60). We set up our playback loudspeaker 10 m inside the subject’s territory, nearest to the boundary with the neighbour that we were simulating

with playback. We chose to place the loudspeaker slightly inside the territory in order to minimize the chance of interference from the neighbour. The loudspeaker occupied the same position for both neighbour and stranger trials. For most birds, we broadcast playback from the same location within each bird's territory across the entire season. In three instances, however, territory borders changed over the course of the experiment. In these instances we moved the speaker to maintain a position 10 m from the edge of the focal bird's territory (average distance moved in these three cases: 5 m). We placed the loudspeaker in a sound baffle made of a 20-inch diameter plastic parabola lined with 2-inch thick foam and a camouflage-coloured fabric. This baffle diminished the noise behind the loudspeaker, in order to further reduce interference from the neighbour. We did not conduct playback to neighbouring birds on the same day; subjects had to be at least one territory apart, and have different neighbours used for playback stimuli to receive playback on the same day.

We carried out playback experiments between 0630h and 1300h. An observer (CM-P) sat at a position 20 m away from the loudspeaker, and dictated the focal bird's behaviour into a microphone to serve as a record of birds' response to playback. Playback trials began once both the focal bird and the neighbouring bird were not singing and when the focal bird was greater than 15 m away from the loudspeaker. The playback period lasted 3 min and was followed by a 5 min post-playback observation period. We focused our analysis solely on the 3-min playback period because most birds began interacting with their neighbours during the post-playback period. After 20 min had elapsed from the end of the first playback trial, the subject received the second

treatment (i.e. neighbour or stranger). We alternated the order of presentation of the neighbour and stranger stimuli, such that each bird received neighbour or stranger playback first an equal number of times.

The design of this experiment necessitated repeated playback to the same animal across different breeding stages. We revisited each bird every 3-7 days to present both neighbour and stranger playback trials. We conducted 5.5 ± 0.24 (mean \pm SE; range 3-7) neighbour and stranger playbacks to each individual.

Fertility

We tracked the breeding stage of focal pairs by observing male and female behaviour and monitoring active nests. We found 17 nests from our 22 subjects (Song Sparrows are secretive nesters, and even with considerable effort we could not find all nests). For the five subjects where we were unable to locate nests, we observed female behaviour to determine breeding stage: if we saw a female with nest material we assumed she was in the nest-building stage; if we saw a female foraging for short periods of time separated by 30-40 mins in the morning we assumed she was incubating. For the 17 subjects with known nests, we used the method outlined in Nice (1943) to back-date nests when we did not have complete data from nest building. We assigned breeding stage length as follows: nest building (2-4 days), egg laying (4-5 days; 4 days for nests with 4 eggs and 5 days for nests with 5 eggs), and incubation (12-13 days). No playbacks took place during the post-incubation period. Sperm storage in Song

Sparrows has not been quantified so we assumed the fertile period for the female to begin six days prior to the laying of the first egg and considered it to end on the day the penultimate egg had been laid (as in Akçay et al., 2012). Since the lengths of breeding stages are not rigidly defined, there were some instances where we were unable to assign birds to a breeding stage during a playback trial (e.g. if we found the nest during incubation and it had been depredated before it hatched); we excluded such trials from analysis.

Sample Size and Statistical Analysis

We excluded several trials from our final analyses due to interference by neighbours, lack of response by focal birds to both neighbour and stranger playback (i.e. the subject was not seen or heard for the duration of either trial), and lack of sufficient information to determine fertility status (see section on Fertility). After exclusion, we had 84 neighbour and stranger trials during the pre-fertile period consisting of 20 focal birds, 62 neighbour and stranger trials during the fertile period consisting of 22 focal birds, and 56 neighbour and stranger trials during the post-fertile period consisting of 20 focal birds.

We conducted principal components analysis (PCA) using SPSS 20.0 (SPSS Inc., Chicago Ill.) to create composite variables that summarized our inter-correlated measurement variables of birds' responses to playback. We included four variables in this analysis: number of flights, closest approach to the loudspeaker, latency to

approach within 10 m of the loudspeaker, and time spent within 10 m of the loudspeaker. We chose to include only physical response variables and not vocal response variables because these are widely recognized to be indicators of aggression in Song Sparrows (Searcy & Beecher, 2009; Searcy et al., 2014). This analysis yielded one principal component factor with an eigenvalue above 1, explaining 69.8% of the variance in behavioural measures, which we retained for analysis (Table 3.1). Principal component 1 (PC1) was positively associated with number of flights and time spent within 10 m of the speaker, and negatively associated with closest approach distance and latency to approach. Accordingly, we interpreted high positive values of PC1 as reflecting high aggression.

To analyze our results, we ran a multiple regression using R (Version 3.2.3, R Core Team, 2016) to compare aggression elicited by neighbour versus stranger playback separately during each period. We used stimulus identity as our predictor variable while controlling for subject identity, number of treatments (i.e. how many times each bird had been subject to playback trials), and order of stimulus presentation (i.e. whether the neighbour treatment was first or second on that day). Following our multiple regression analysis we confirmed that the residuals of our analyses were normal using q-q plots and constructed plots of our residuals to confirm the data were homoscedastic.

Results

Male song sparrows displayed a dear enemy effect through increased aggression toward strangers versus neighbours during their female's pre-fertile (ANOVA: $F = 9.0$, $p =$

0.004; Table 3.2, Fig. 3.1) and post-fertile periods (ANOVA: $F = 7.9$, $p = 0.009$; Table 3.2, Fig. 3.1). During the female's fertile period, however, male song sparrows responded similarly to neighbour and stranger playback (ANOVA: $F = 0.4$, $p = 0.55$; Table 3.2, Fig. 3.1) as a result of increased aggression toward neighbours.

Discussion

We found that flexibility in the dear enemy effect is influenced by female fertility in male Song Sparrows. Male Song Sparrows displayed a dear enemy effect when their female was in the pre-fertile and post-fertile stages, but did not display the dear enemy effect when their female was fertile. These results are consistent with our prediction that male Song Sparrows would increase aggression toward neighbours during periods of female fertility, presumably to protect their paternity.

The difference in expression of the dear enemy effect across different breeding stages suggests that male Song Sparrows exhibit a mate-guarding tactic wherein they increase aggression toward neighbours during periods when extra-pair paternity is an increased risk. Indeed, Song Sparrows have moderately high rates of extra-pair paternity and neighbours are most often the sires of extra-pair offspring in this species (e.g. Hill et al., 2001). Therefore, guarding against paternity loss to rival neighbouring males is worthwhile for male Song Sparrows. It is possible that increased aggression toward neighbours in Song Sparrows ensures a male's paternity through discouraging the neighbouring male from intruding, and possibly by demonstrating the male's quality to

its mate. In another temperate songbird, the black-capped chickadee (*Poecile atricapillus*), females had more extra-pair copulations if their high-ranking mate lost a singing contest with a playback-simulated male during her fertile period than if their high-ranking mate won such a singing contest (Mennill et al., 2002). Thus it benefits a male bird to respond strongly to rival males during their female's fertile period and win contests in order to ensure their paternity. However, during periods when the female is not fertile, a male may benefit by foraging, building nests, or provisioning young rather than engaging in costly aggressive behaviour with a neighbour. Future work could include a genetic analysis of whether male Song Sparrows that are more aggressive to neighbours during their female's fertile periods succeed by suffering fewer extra-pair offspring in their nest.

During the pre-fertile period, male birds establish territories and negotiate boundaries with neighbours. Neighbouring males should act aggressively toward each other until boundaries are firmly established (Briefer et al., 2008). This is likely the case very early in the breeding season after birds arrive from their wintering grounds. It is important to note that our study began roughly 2-3 weeks after Song Sparrows had arrived on their breeding grounds from migration; by this time territories appeared to be stable and we did not observe aggressive encounters between established neighbours. During the fertile period, Song Sparrows engage in nest-building and copulations with their mate (Nice, 1943), and thus mate-guarding to ensure paternity is important during this time. During the post-fertile period (defined as the incubation period in our study), territories are well-established and neighbours have little reason to display aggression

toward each other, as they do not generally pose a threat to a territory or to paternity. In fact, males may be attempting to attract neighbouring females during this time period to seek out extra-pair copulations (Foote & Barber, 2009). Various factors such as recent intrusions (Akçay et al., 2009), relatedness (Akçay et al., 2013), and male quality (Sandoval, 2011) can affect male response to neighbours, however female fertility appears to also play an important role.

Eliassen and Jørgensen (2014) hypothesized that by engaging in extra-pair copulations, females create a cooperative neighbourhood, wherein males are incentivized to cooperate with nearby males because a male may have extra-pair offspring in his neighbours' nests. Females then benefit from this if males engage in more cooperative behaviours, and fewer aggressive interactions. From this hypothesis, they suggest that males should display a dear enemy effect when females are not fertile. Our results are consistent with this idea as we found that male Song Sparrows show a dear enemy effect when their female is not fertile, but do not show a dear enemy effect when their female is fertile. However, it is important to note that this evolution of a cooperative neighbourhood by extra-pair copulations incurs a cost through a period of time (i.e. during the fertile period) when neighbours are very aggressive and uncooperative toward each other in order to protect their own paternity. Alternatively, males may reduce aggression to each other during non-fertile periods because males can only secure extra-pair copulations during neighbouring females' fertile periods. As a result, high aggression during non-fertile periods provides a substantially lower benefit than high aggression during the fertile period. The cooperative neighbourhood

hypothesis also fails to explain why males display a dear enemy effect during the pre-fertile period, when extra-pair fertilizations are not yet possible.

We developed a visual model of the relative threat of neighbours versus strangers over the course of a female's breeding period. We speculated that neighbours and strangers would pose similar levels of threat during the female fertile period. This position was supported by our results given that male Song Sparrows responded equally aggressively to neighbour and stranger playback during this period. However, a more comprehensive method of examining the true relative threat levels of neighbours and strangers during this time period may be through an experimental design similar to Stoddard et al. (1990), wherein male Song Sparrows were presented with neighbour and stranger playback simultaneously and the observers noted the amount of time males spent near each speaker. This design forces males to choose which stimulus to respond to and may elucidate which class of conspecifics are more threatening during this time period. If strangers are a higher threat during the fertile period, we would expect males to focus their response on stranger playback; if neighbours are a higher threat during this time period we would expect males to focus their response on neighbour playback; if neighbours and strangers were an equal threat during this period we would expect males to focus their response on both playbacks.

The results from this study have implications for the experimental design of future investigations of the dear enemy effect. It is important for researchers to take breeding stage of subjects into consideration when conducting dear enemy effect studies or, by extension, any studies involving neighbour playback. As shown here, a

study conducted during an animal's fertile period may yield a negative result even though these animals may display the dear enemy effect during the non-fertile periods. In conjunction with Briefer et al. (2008) and Courvoisier et al. (2014), our study provides evidence that the social interactions between territorial birds are dynamic over a breeding season. The dear enemy effect appears to be a fluid phenomenon that is partially determined by the breeding stage of females.

Conclusions

Song Sparrows displayed a dear enemy effect during the pre-fertile and post-fertile period but not during the fertile period. Our results suggest that Song Sparrows respond more strongly to neighbours during their female's fertile period as a mate-guarding tactic to reduce extra-pair paternity. Future work could investigate whether males that are more aggressive to neighbours during their female's fertile periods have fewer extra-pair offspring in their own nest.

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Tables and Figures

Table 3.1. Principal component factor loadings for the first principle component factor, which we refer to as “male aggression score”. This factor had an eigenvalue of 2.8 and explained 69.8% of the variance observed. It was the only principal component with an eigenvalue above 1.

Variables	PC1
Closest approach	-0.86
Latency to approach within 10 metres	-0.92
Time spent within 10 metres	0.79
Number of flights	0.76
Eigenvalue	2.80
Percent Variance Explained	69.8%

Table 3.2. Male Song Sparrows varied expression of the dear enemy effect with their female's fertility. We analyzed these data using a multiple regression.

	Degrees of Freedom	F	P
Pre-Fertile			
Stimulus Identity	1	9.0	0.004
Focal Bird	19	1.5	0.14
Order	1	0.2	0.69
Number of Treatments	1	2.8	0.10
Fertile			
Stimulus Identity	1	0.36	0.55
Focal Bird	21	3.9	0.0001
Order	1	1.7	0.20
Number of Treatments	1	0.007	0.93
Post-Fertile			
Stimulus Identity	1	7.9	0.009
Focal Bird	18	7.6	<0.0001
Order	1	0.51	0.48
Number of Treatments	1	2.2	0.15

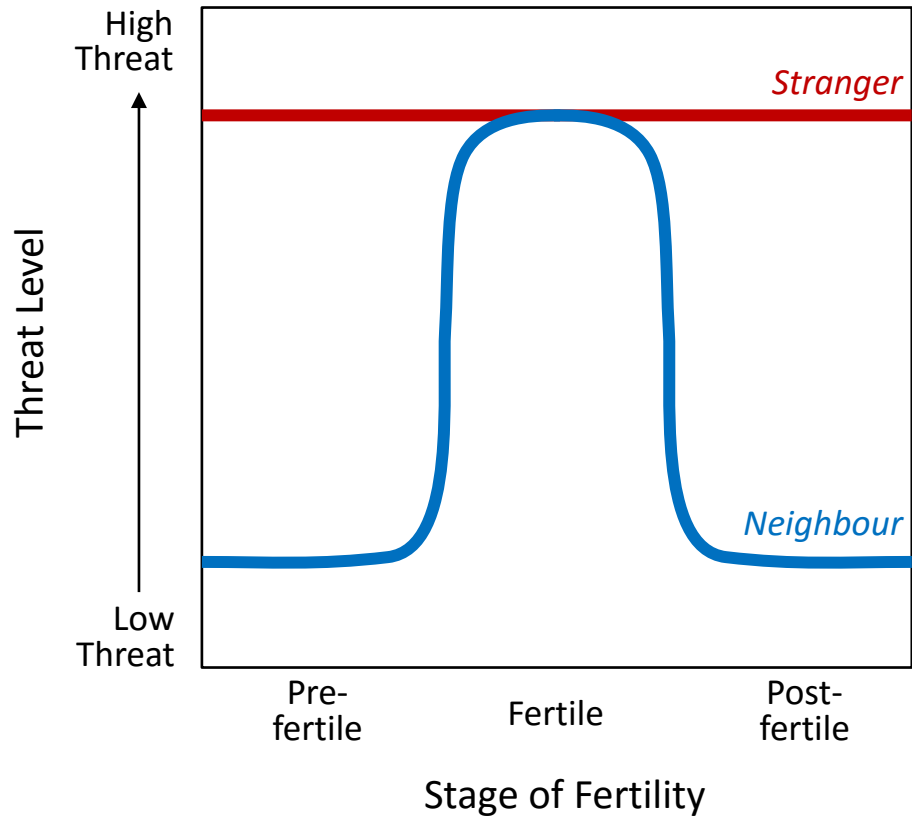


Figure 3.1. Visual model representing the relative threat posed by stranger males versus neighbour males to a territorial male, in relation to his partner's stage of fertility. Stranger males represent a constant threat to a territorial male over a breeding season because they always threaten a male's territory ownership. Neighbouring males, on the other hand, do not represent a threat to territory ownership after territories are established; instead they only represent a threat when a territorial male's partner is fertile because it can engage in extra-pair copulations with a male's mate.

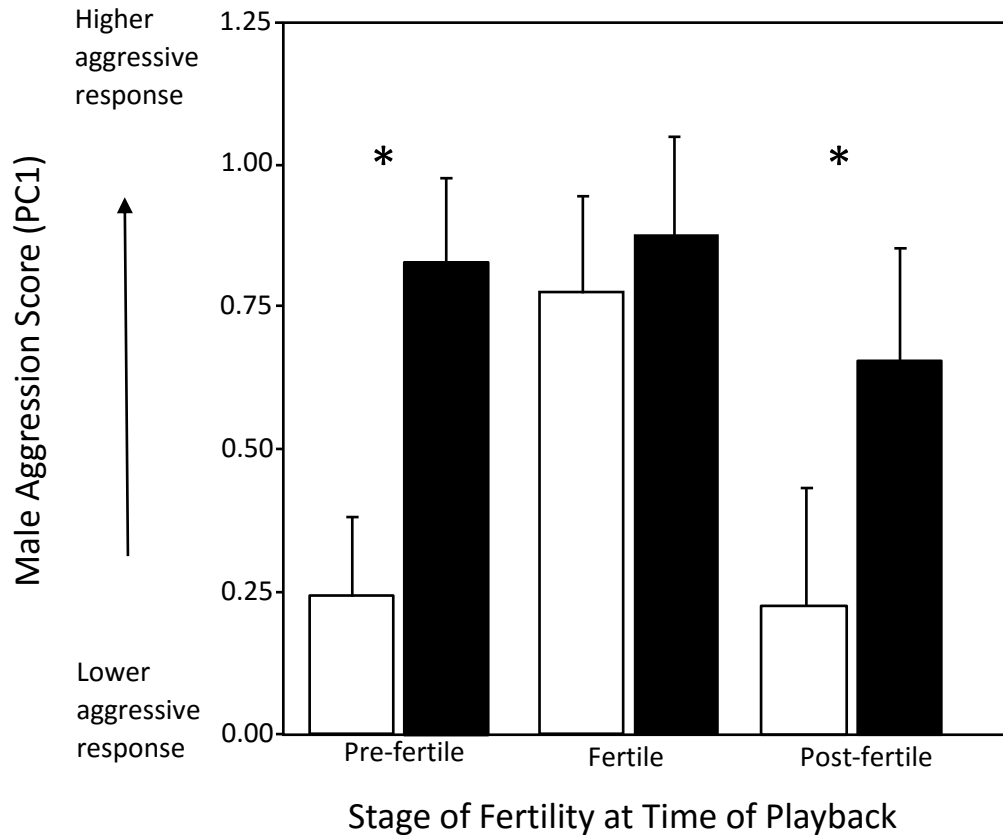


Figure 3.2. Male Song Sparrows displayed the dear enemy effect during their female’s pre-fertile period and post-fertile period but not during her fertile period. Response to neighbours is represented in white and response to strangers is represented in black. Male aggression scores are a principal component score that summarizes four behavioural measurements of birds’ responses to playback. Data are presented as means \pm standard errors.

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CHAPTER 4

MALE SONG SPARROWS HAVE ELEVATED TESTOSTERONE IN RESPONSE TO PLAYBACK OF NEIGHBOURS VERSUS STRANGERS

Chapter Summary

Upon hearing a conspecific signal, animals must assess their relationship with the signaller and respond appropriately. Territorial animals usually respond more aggressively to strangers than neighbours in a phenomenon known as the “dear enemy effect”. This phenomenon likely evolved because strangers represent a threat to an animal’s territory tenure and parentage, whereas neighbours only represent a threat to an animal’s parentage, given that they already possess a territory. Although the dear enemy phenomenon has been widely documented using behavioural response variables, little research has been conducted on the physiological responses of animals to neighbours versus strangers. We sought to investigate whether the dear enemy effect is observed physiologically by exposing territorial male Song Sparrows (*Melospiza melodia*) to playback simulating a neighbour or a stranger, and then collecting blood samples to measure plasma testosterone levels. We predicted that Song Sparrows would have increased testosterone levels after exposure to stranger playback compared to neighbour playback, due to the role testosterone plays in regulating aggression. Contrary to our prediction, we found that Song Sparrows had higher testosterone levels after exposure to neighbour playback compared to stranger playback. We discuss several explanations for our result, notably that corticosterone may regulate the dear enemy effect in male Song Sparrows and this may inhibit plasma testosterone. Future studies will benefit from examining corticosterone in addition to testosterone, to better understand the hormonal underpinnings of the dear enemy effect.

Introduction

Territorial animals interact with many types of conspecific individuals. Upon encountering a conspecific individual, an animal must assess its threat level and respond appropriately. Across a wide range of animal taxa, territorial interactions are more intense when a resident animal encounters an unfamiliar stranger versus a familiar neighbour (Temeles, 1994). This phenomenon is known as the “dear enemy effect” (Fisher, 1954). Strangers might be more of a threat to territory takeover than neighbours who, by definition, already possess a territory and therefore might be less likely to usurp the resident animal’s territory (Getty, 1987). Because neighbours may be less of a threat, the establishment of a less aggressive relationship between neighbours allows individuals to maximize their fitness by focusing effort on activities such as foraging and provisioning for young instead of costly aggressive encounters. The dear enemy effect has been reported in many animal taxa including birds (e.g. Hardouin et al., 2006), mammals (e.g. Rosell et al., 2008), insects (e.g. Langen et al., 2008), frogs (Lesbarrères & Lodé, 2002), lizards (Whiting, 1999), fish, (McGregor & Westby, 1992), and crustaceans (Booksmythe et al., 2010).

The dear enemy effect has been well studied using physical and acoustic measures of behavioural response (Temeles, 1994). In contrast, physiological responses to neighbour and stranger stimuli are almost completely unknown (although see Aires et al., 2015). Under the Challenge Hypothesis, territorial male animals are predicted to increase their testosterone levels in response to a territorial challenge (Wingfield et al.

1990). Testosterone is a steroid hormone that often regulates aggression in vertebrates. For example, experimentally increased testosterone causes increased aggression toward conspecific individuals (e.g. Wingfield, 1994; Mougeot et al. 2005) and testosterone levels often increase during simulated territorial intrusions (e.g. Hau et al. 2000; Wingfield & Wada 1989). Given that testosterone modulates responses to territorial challenges, and given that strangers might pose a heightened threat in comparison to neighbours, we predict that individuals will increase circulating testosterone levels when exposed to a stranger more than when exposed to a neighbour. This increase in testosterone may mediate the behavioural responses observed in dear enemy effect studies. Only one study has previously examined this potential hormonal mechanism of the dear enemy effect by examining a tropical fish species; Mozambique tilapia (*Oreochromis mossambicus*) showed an increased level of 11-ketotestosterone in response to presentation of a stranger in comparison to a neighbour (Aires et al. 2015). It is unknown whether any other vertebrates have a similar endocrine response to challenges from neighbours versus strangers.

We used song playback to study the role of testosterone in mediating the dear enemy effect in territorial male Song Sparrows (*Melospiza melodia*). We exposed territorial males to playback simulating either an unfamiliar stranger or an established territorial neighbour and then sampled their testosterone levels, to test the prediction that birds would show heightened testosterone during confrontation with a stranger. Song Sparrows are an ideal study system for this experiment given that multiple previous studies show that they display the behavioural response predicted by the dear

enemy hypothesis (Chapter 3; Harris & Lemon 1976; Kroodsma 1976; Stoddard et al. 1990; Stoddard et al. 1991) and because they increase testosterone when challenged by conspecifics during breeding (Wingfield & Wada 1989).

Methods

Study Site and Species

We presented territorial male Song Sparrows with experimental playback of recordings of conspecific song at the Queen's University Biological Station (44° 34' N, 76° 19' W; Ontario, Canada). During April of 2016 we recorded birds and mapped territories by spending at least 90 minutes over multiple days observing each bird and logging his song posts into a GPS (Garmin 60CS). From May 4 to May 19 2016 we conducted playback experiments on 22 mated male Song Sparrows. Four birds were banded from studies in previous years, and we banded 12 of the birds we caught in 2016 with unique combinations of colour bands and a Canadian Wildlife Service numbered band. We had no difficulty identifying the six unbanded birds based on location, behaviour, and individually distinctive songs (song sharing is low in Eastern populations of Song Sparrows; Hughes et al., 2007; Stewart & MacDougall-Shackleton 2008; although see Foote & Barber 2007).

Playback Stimuli

To construct playback stimuli we recorded spontaneous bouts of singing from male Song Sparrows. We used an omni-directional microphone (model: Sennheiser ME62/K6) mounted inside of a parabolic reflector (model: Telinga MK2) connected to a solid-state digital recorder (model: Marantz PMD660, 44.1 kHz sampling rate, 16-bit encoding, WAVE format). We collected five song types from each male to use in constructing playback stimuli. We isolated five songs that showed no overlapping background noise (assessed visually based on spectrograms generated in Audition 3.0, Adobe, San Jose, CA), and we normalized each song to -1 dB using the “normalize” feature of Audition 3.0. Each stimulus was 30 minutes in length and consisted of five song types played at a rate of one song every 10 seconds (this is a natural singing rate for this population based on our preliminary observations). One song type was broadcast for 3 minutes before switching to a different song type (this too was within the range of natural song type switching in our population). We cycled through each of the five song types twice for a total of 30 minutes (Figure 4.1). Each stimulus set was composed of different song types from the same bird.

We collected neighbour stimuli from a bird adjacent to each playback subject. We collected stranger stimuli from a bird at least 2 km away from the focal bird. We had 14 stimuli in total: four were used twice (three were used once as a neighbour and once as a stranger, one was used twice as a stranger) and two were used three times (twice as a neighbour and once as a stranger).

In addition to neighbour and stranger stimuli used during the playback phase of the experiment, we also created a capture stimulus for the capture phase of the experiment (see below). A separate separate stimulus was necessary because the subjects' responses to neighbour playback did not incite sufficiently close approaches to permit capture. Songs for the capture stimulus were collected from a bird more than 5 km away from all of our playback subjects. We designed the capture stimulus to be highly aggressive, inciting rapid, close approach to the loudspeaker so that we could capture birds in mist nets. The stimulus consisted of five song types from the same bird, each played five times separated by 2 seconds of silence, and with 8 seconds of Song Sparrow calls inserted prior to each change in song type. We used the same capture stimulus for all birds.

Playback Experiment

We presented each of the 22 subjects with 30 minutes of stranger playback (N=10) or neighbour playback (N=12) (Fig. 4.1). After this 30-minute “playback phase” we captured the focal bird using playback of the capture stimulus, i.e. the “capture phase”, which were broadcast for as long as necessary to capture the bird: on average the capture stimulus was played for 3.7 ± 0.6 minutes (mean \pm SE; range: 0.6 – 9.0 minutes). The amount of time the capture stimulus was played did not differ between neighbour and stranger trials (Exact Wilcoxon Rank Sum Test: $W = 63$, $p = 0.86$, $N = 22$). We collected a blood sample for testosterone analysis following capture (see *Hormone Analysis* for details on time course of testosterone sampling). Each subject received

either a stranger or a neighbour playback (i.e. a between-subjects design). We played either neighbour or stranger playback from the loudspeaker, determined randomly based on an initial coin toss followed by alternation of neighbour and stranger playback.

All playback trials took place between 0630 and 1130 h. Before starting our playback trials, we set up a mist net within the subject's territory but kept it closed until the capture phase. We set up a loudspeaker (model: Scorpion TX200, FoxPro Inc.) which we positioned 10 m from the edge of the territory of the focal bird (as determined during territory mapping), near the boundary of the neighbour used for the playback stimulus. We placed the loudspeaker within a sound baffle constructed from a 20-inch diameter plastic parabola with a 2-inch thick lining of foam covered by camouflage fabric. This baffle appreciably reduced noise behind the speaker with the aim to reduce interference from neighbours. We did not observe any interactions between neighbours and focal birds, and neighbours never appeared to respond to playback. We attempted to minimize the effect of breeding stage on playback responses by conducting playback experiments during a short time span (15 days) when we expected that many of the pairs in the population were incubating eggs. We confirmed that two subjects were in the incubation stage, and we found one female with an enlarged brood patch which supports our assumption that she was incubating.

We measured the birds' aggressive responses to playback by assigning each individual an aggression score (Emlen 1971; Wunderle 1978) between 1 and 5. A higher score was assigned to more aggressive responses (e.g. spending time near the speaker, making a high number of flights around the speaker, spending more time responding to

the stimulus, singing more soft songs) and a low score was assigned to a less aggressive response. If a bird responded rapidly to playback (within 1 min) and spent a large amount of time during the playback period (≥ 20 mins) within 15 m of the speaker we gave it a score of 5. We gave lower scores to birds who had a higher latency to respond to playback or spent less time responding to playback. A bird received a score of 1 if it did not approach to within 15 meters of the speaker and did not engage in any aggressive behaviour. We did not consider normal-amplitude song when creating aggression scores because these are poor predictors of aggressive intent in Song Sparrows (Searcy & Beecher 2009; Searcy et al. 2014). Although this method of quantifying behavioural response is qualitative, it helped us to determine whether a dear enemy effect was observed across our trials as has been found in several other studies of Song Sparrows (Chapter 3; Harris & Lemon 1976; Kroodsma 1976; Stoddard et al. 1990; Stoddard et al. 1991).

Hormone Analysis

We collected blood samples for hormone analysis immediately upon extracting birds from mist nets during the capture phase. We collected up to 75 μL of blood from the brachial vein using a 26-gauge needle and heparinized microcapillary tubes. Blood samples were immediately stored on ice until they were centrifuged and the plasma was harvested within 5 hours of blood collection. Separated plasma samples were stored at -20°C until they were assayed 4-5 weeks later. Blood samples were collected on average within 7.8 ± 0.5 min (mean \pm SE; range: 3.6 - 13.0 min) of the end of the playback phase

and on average 4.0 ± 0.4 min (mean \pm SE; range: 1.5 – 7.3 min) following capture. The amount of time to collect blood samples did not differ between neighbour and stranger trials (Student's T-test: $t = 0.76$, $p = 0.46$, $N = 22$). Testosterone does not show an increase in Song Sparrows until exposure to at least 10 minutes of playback (Wingfield & Wada, 1989). We collected blood from only three birds after 10 minutes (two birds caught after 10.5 minutes and one bird caught after 13 minutes) but found no effect of time before blood collection on plasma testosterone (Spearman's rank correlation: $\rho = -0.16$, $p = 0.48$). Exclusion of the three data points collected after 10 minutes provided the same result as those presented below, and therefore we retained them in our final analysis.

We used a commercially available enzyme immunoassay kit to quantify plasma testosterone (1-2403, Salimetrics, State College, PA, U.S.A.) that had been previously validated for Song Sparrows in our study population (see Schmidt et al. 2014). The antibody of this kit has some cross reactivity with androgens, thus hereafter we refer to "androgen levels" rather than "testosterone levels". We used assay buffer to dilute plasma 1:12. We then analyzed samples in duplicate, using 25 μ L of the diluted plasma in each well. All samples were run in one assay. The intra-assay coefficient of variation was 13.2% for a low control (250 pg/mL), and 1.5% for a high control (2500 pg/mL). The total intra-assay coefficient of variation was 4.9%. Androgen levels of all samples fell within the range of the standard curve (6.1-600 pg/ml).

Statistical Analysis

We used R (Version 3.2.3, R Core Team, 2016) to analyze our data. Because the androgen data were not normal (Shapiro-Wilk Normality Test: $W = 0.69$, $p < 0.0001$, $N = 22$) we used a Wilcoxon rank sum test to analyze the data, with treatment group as a predictor variable. Similarly because the aggressive response data were not normal (Shapiro-Wilk Normality Test: $W = 0.83$, $p = 0.001$, $N = 22$) and because the data had ties, we used an exact Wilcoxon rank sum test using the package `exactRankTests` (Hothorn & Hornik, 2015) to analyze the data with treatment group as a predictor variable. Lastly, due to the non-normality of the data, we conducted a Spearman's rank correlation to determine if there was a correlation between plasma androgens and aggression score.

This study was approved by the University of Windsor Animal Care Committee under AUPP-16-01 and by the Queen's University Animal Care Committee under permit 2016-1652.

Results

Song Sparrows had higher plasma androgens when exposed to neighbour playback than when exposed to stranger playback (Wilcoxon rank sum test: $W = 100$, $p = 0.007$; Fig. 4.2). Plasma androgen levels were more than twice as high, on average, among birds receiving neighbour playback versus birds receiving stranger playback.

Song Sparrows showed higher aggression scores when exposed to stranger playback than when exposed to neighbour playback (exact Wilcoxon rank sum test: $W = 7.5$, $p = 0.003$; Fig. 4.3), consistent with the dear enemy effect.

Across treatment groups, Song Sparrows with lower plasma androgens had higher aggression scores (Spearman's rank correlation: $\rho = -0.50$, $p = 0.02$).

Discussion

We found that territorial male Song Sparrows exhibited lower androgen levels in response to playback of a stranger versus playback of a neighbour. Similar to previous work on Song Sparrows (Chapter 3; Harris & Lemon, 1976; Kroodsmma, 1976; Stoddard et al., 1990; Stoddard et al., 1991), we did observe a behavioural dear enemy effect in our study where males responded more strongly to playback of strangers versus neighbours. Androgen levels and behavioural responses were negatively correlated with each other, indicating that Song Sparrows that displayed higher levels of aggression had decreased levels of testosterone. This finding stands in direct contrast to our prediction that strangers, which might be perceived as higher threat individuals, would induce higher testosterone levels than neighbours. Strangers should pose a higher threat than neighbours because they theoretically represent a higher threat to territory takeover than neighbours (Temeles, 1994). This dichotomy in threat levels is reflected in an increased behavioural response to strangers reported here as well as in other studies of

Song Sparrows (Chapter 3; Harris & Lemon, 1976; Kroodsma, 1976; Stoddard et al., 1990; Stoddard et al., 1991).

The Challenge Hypothesis (Wingfield et al., 1990) predicts that territorial animals exposed to a social challenge should increase plasma testosterone to regulate aggression. However, due to the negative effects of testosterone (e.g. reduced parental care; Wingfield et al., 2001), animals may benefit from increased testosterone levels only when the benefits outweigh the costs. Across the literature, some studies report an increase in testosterone during simulated intrusions (e.g. Wikelski et al., 1999; Wingfield & Hahn, 1994; Wingfield & Wada, 1989), however, other studies report an absence of testosterone response during simulated intrusions (e.g. Deviche et al., 2014; Gill et al., 2008; Landys et al., 2010; Rosvall et al., 2012) or a decrease in plasma testosterone during simulated intrusions (e.g. Landys et al., 2007; Van Duyse et al., 2004) possibly due to the detrimental effects of testosterone on breeding effort through reduced parental care. We found that exposure to a higher threat (i.e. playback simulating a stranger) resulted in lower plasma androgens than exposure to a lower threat (i.e. playback simulating a neighbour) during a period when all subjects were assumed to be incubating. This suggests that plasma androgens do not play a role in dear enemy effect expression during incubation in Song Sparrows, and that Song Sparrows may benefit from avoiding the detrimental effects of testosterone during this time period. Indeed, plasma testosterone appears to be lower during incubation in Song Sparrows (Wingfield & Hahn, 1994), and plasma androgens following either playback in our study are relatively low compared to plasma testosterone following challenges during the pre-

nesting phase in other studies on Song Sparrows (Wingfield, 1985; Wingfield & Wada, 1989).

Given that higher testosterone is associated with reduced parental care (Schoech et al., 1998; Silverin, 1980; Van Roo, 2004), Song Sparrows may mount an aggressive response through mechanisms other than testosterone during their incubation and provisioning stages. Wingfield (1984) gave Song Sparrows testosterone implants and measured the testosterone levels of their neighbours over a breeding season; during incubation and provisioning, Song Sparrows with testosterone-implanted neighbours had the same testosterone levels as Song Sparrows with control neighbours, whereas in other breeding stages Song Sparrows with testosterone implanted neighbours had higher testosterone levels than controls. This suggests that during incubation and provisioning, testosterone plays less of a role in interactions with aggressive conspecific individuals. In addition to reducing parental care, increased testosterone levels can be associated with reduced immune function, reduced fat stores, and increased mortality (reviewed in Wingfield et al., 2001) which Song Sparrows would benefit from avoiding. It is possible that instead of testosterone, corticosterone drives dear enemy effect expression in Song Sparrows during incubation. Corticosterone is the major glucocorticoid in birds and plays a role in regulating stress responses through the mobilization of resources such as glucose to cope with energy-demanding activities (Romero, 2002), which may include intense response to conspecific stimulus.

Landys et al. (2007), Van Duyse et al. (2004), and Sorenson et al. (1997) found that upon exposure to conspecific stimuli, their subjects (birds in all studies) responded

with increased aggression and corticosterone levels, but decreased testosterone levels. Additionally, female Song Sparrows exposed to a simulated intrusion had decreased testosterone levels compared to controls (Elekovich & Wingfield, 2000) and male Song Sparrows increased corticosterone when exposed to a simulated territorial intrusion in the mid-late breeding season (Newman & Soma, 2011; but see Wingfield & Hahn, 1994). Corticosterone is well established as a suppressor of the hypothalamic-pituitary-gonadal (HPG) axis and can have an inhibitory effect on plasma testosterone (Deviche et al., 2010; Dong et al., 2004; James et al., 2008; McGuire & Bentley, 2010; Monder et al., 1994). Landys et al. (2007) suggest that corticosterone may suppress testosterone levels during challenges in order to ensure testosterone levels remain low and do not interfere with parental care, although they only apply this hypothesis to birds that raise only a single brood of offspring each year, whereas Song Sparrows at our field site are multi-brooded. Unfortunately, we were unable to test whether corticosterone was associated with the dear enemy effect in our study due to lack of samples collected under 3 minutes from capture (corticosterone sampling requires blood samples to be collected within this timeframe; e.g. Dawson & Howe 1983) and lack of plasma following testosterone analyses. Future studies may benefit from including corticosterone levels as well as testosterone levels when examining the physiological responses associated with the dear enemy effect. Future studies may also benefit from conducting experiments during different breeding stages.

An alternative to the idea that corticosterone mediates the dear enemy effect is that dehydroepiandrosterone (DHEA) may instead regulate the dear enemy effect. DHEA

is a steroid hormone and is metabolized to testosterone or 17- β -estradiol (E2) in the brain (Heimovics et al., 2016). DHEA has been associated with aggression in non-breeding Song Sparrows despite the suppression of testosterone during this time period (Newman & Soma, 2011; Soma et al., 2002) suggesting that it may mediate behavioural responses to conspecific challenges. Furthermore, E2 has been shown to regulate aggression in response to simulated territorial intrusions in non-breeding male Song Sparrows (Soma et al., 2000). However, this hypothesis fails to explain why plasma androgens in response to strangers were lower than in response to neighbours because there is no evidence that DHEA or E2 suppress plasma testosterone, and increased DHEA may be associated with increased plasma testosterone (Soma et al., 2002). Nevertheless, it may be beneficial to include neurosteroids such as DHEA and E2 in future studies investigating the hormonal correlates of the dear enemy effect alongside corticosterone.

Another possible explanation for our results is that during neighbour playback, subjects may have had visual contact with their neighbour, whereas during stranger playback subjects would not have seen the simulated intruder. Wingfield and Wada (1989) showed that Song Sparrows increase testosterone levels most when an acoustic stimulus is paired with a visual stimulus. Song Sparrows in our study that were exposed to neighbour playback may have had both an acoustic and visual stimulus whereas subjects exposed to stranger playback only had exposure to an acoustic stimulus. We did not observe any interactions between focal birds and neighbours during our trials, however we were not able to continuously track the focal bird's location if the bird temporarily left the playback area. Furthermore, Wingfield & Wada (1989) found an

increase in plasma testosterone after only 10 minutes of playback; our study measured plasma androgens after roughly 40 minutes from the beginning of playback. It is possible that Song Sparrows had increased levels of plasma androgens during the middle of stranger playback, which decreased when no stranger was observed visually, whereas during neighbour playback, visual confirmation of the neighbouring male may have caused higher androgen levels to persist. Future studies would benefit from either removing neighbouring Song Sparrows during neighbour playback trials or capturing neighbours and strangers to use as live decoys during playback trials, although the logistics of this may prove challenging.

Lastly, stranger playback may have induced a “loser effect” in our study, wherein Song Sparrows decreased testosterone levels having thought they lost the challenge. This effect has been found in other species (e.g. Hirschenhauser et al., 2013; Johnsen & Zuk, 1995) where winners of a social challenge increased testosterone more than losers following a challenge. However a study in black redstarts (*Phoenicurus ochruros*) did not find any association of testosterone with a simulated winner or loser effect (Apfelbeck et al., 2011). Furthermore, a study on Song Sparrows found no evidence of a loser effect after a 30 min playback (Wingfield & Wada, 1989) and it is improbable that stranger playback would induce a loser effect whereas neighbour playback would not, thus this explanation is unlikely.

Conclusions

Song Sparrows increased androgen levels more in response to neighbour playback than in response to stranger playback despite displaying a dear enemy effect. Our results suggest that androgens do not play a role in regulating the aggression observed during dear enemy effect expression in the incubation breeding stage in Song Sparrows. Alternatively, the dear enemy effect may be mediated by corticosterone during this time.

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Tables and Figures

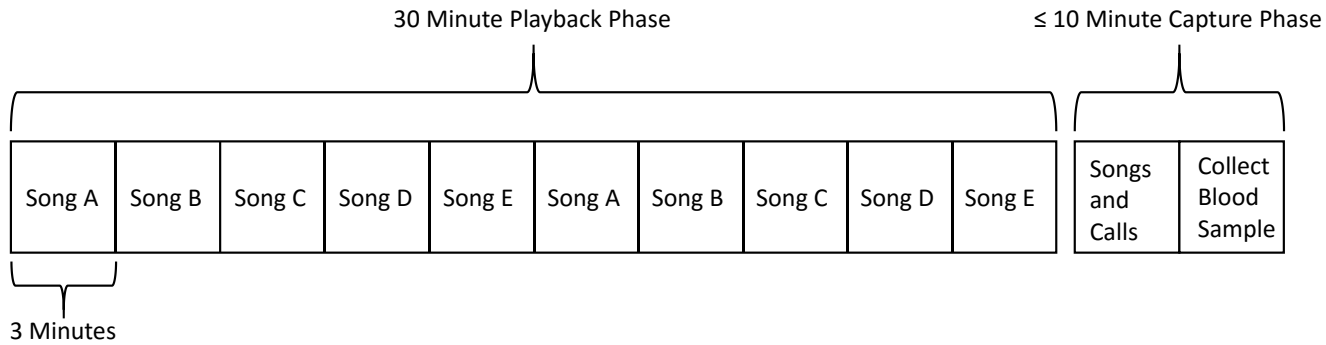


Figure 4.1. Song Sparrows received 30 minutes of playback consisting of either neighbour or stranger songs, with each song type played for 3 minutes. Five song types were used in total for each playback stimulus and each song type was repeated twice. Following the 30 minute playback, we played our capture stimulus that consisted of Song Sparrow songs and calls to facilitate quick capture of the subject and we collected a blood sample from the subject within 10 minutes (except in three cases, see *Hormone Analysis*).

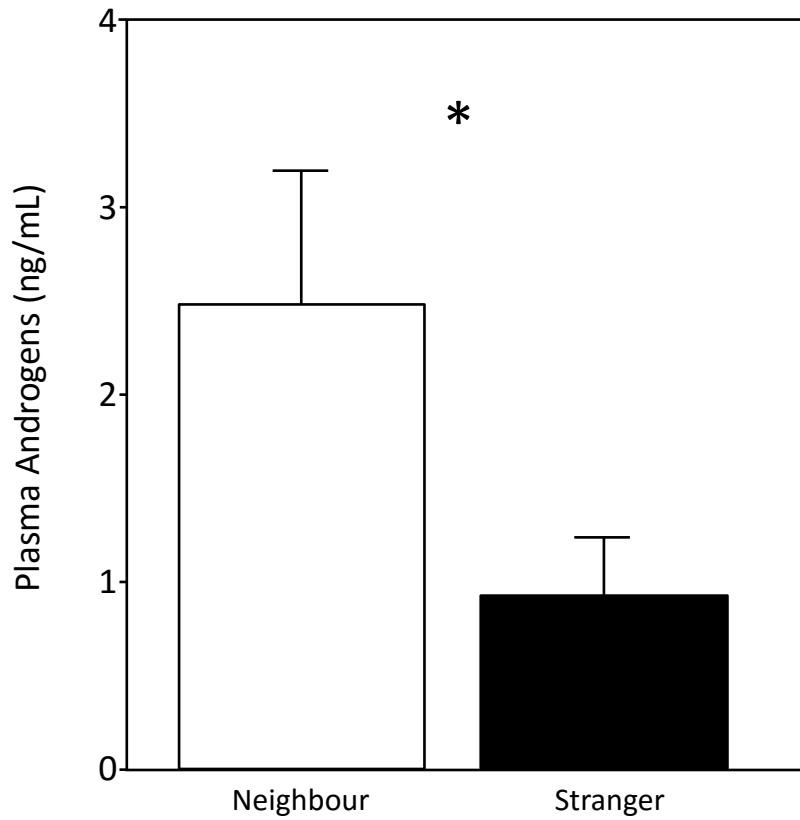


Figure 4.2. Territorial male Song Sparrows had higher levels of plasma androgens after 30 minutes of playback of recorded songs of a neighbouring male (N=12) compared to 30 minutes of playback of recorded songs of an unfamiliar stranger (N=10). These trend was significant at a p-value of 0.007 (Wilcoxon rank sum test). Data are presented as means \pm SE.

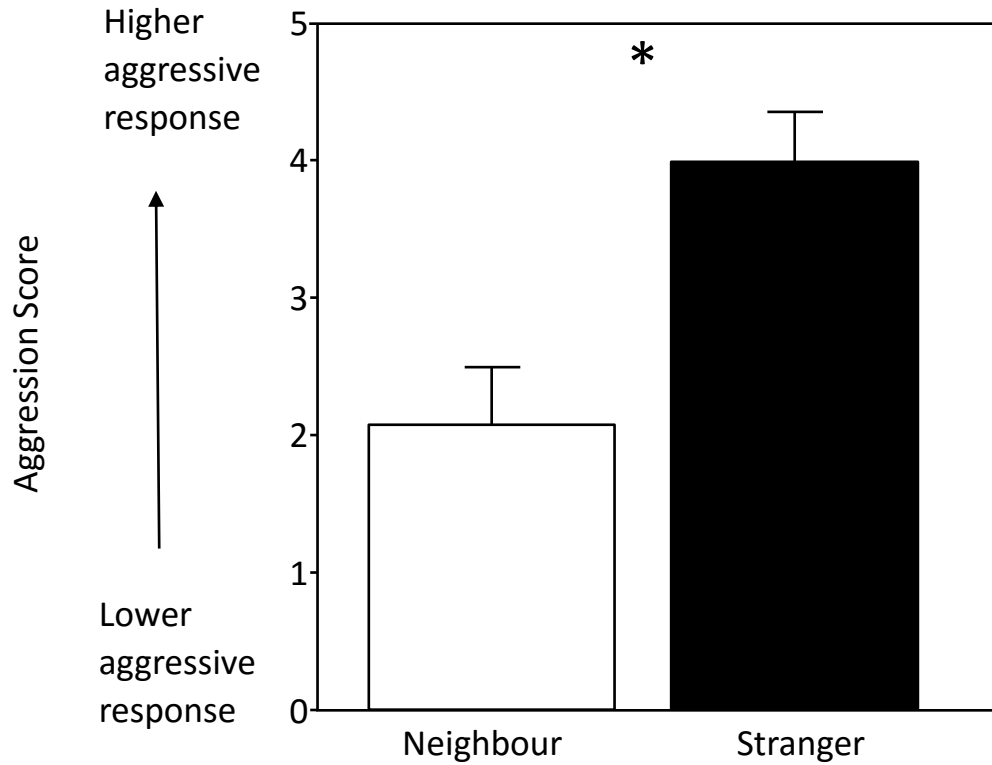


Figure 4.3. Territorial male Song Sparrows showed higher aggression scores (a qualitative measurement of birds' responses to playback) when exposed to playback of recorded songs of a stranger male (N=12) compared to playback of recorded songs of a neighbouring male (N=10), consistent with the dear enemy effect. This was significant at a p-value of 0.003 (exact Wilcoxon rank sum test). Data are presented as means \pm SE.

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CHAPTER 5

GENERAL DISCUSSION

The ability to accurately assess and respond to another animal's threat level allows an animal to act appropriately during different social interactions. Since neighbours and strangers represent different threat levels, animals may optimize their fitness by responding differently to these two classes of individuals. By only engaging in aggressive behaviours with high threat individuals, animals can spend more time and energy on activities such as foraging or provisioning young. Furthermore, being able to assess a rival's changing threat level over different time periods allows a finer tuning of response; animals can mount a more aggressive response during periods when the rival is a higher threat and mount a less aggressive response during periods when the rival is a lower threat. Most research has focused on the ability of certain animals to display neighbour-stranger discrimination and the dear enemy effect. Little research has investigated the underlying factors that drive the dear enemy effect. In this thesis, I explored the constraints and drivers of neighbour-stranger discrimination and the dear enemy effect, expanding our understanding of social interactions in territorial animals.

In Chapter 2 I investigated if large song repertoires constrain neighbour-stranger discrimination. Early research found evidence that suggested large repertoires constrain neighbour-stranger discrimination (Falls & D'Agincourt 1981; Godard, 1993; Harris & Lemon 1976; Kroodsma, 1976; Searcy et al. 1981), however other studies have found neighbour-stranger discrimination in large-repertoire species (Briefer et al. 2008; Hwang & Park, 1993). Thus, it is unclear whether large repertoires constrain neighbour-stranger discrimination. I tested this hypothesis using a neighbour-stranger playback experiment with a large-repertoire songbird, the Red-eyed Vireo (*Vireo olivaceus*), and a

phylogenetically-controlled comparative analysis across 34 songbirds. Red-eyed Vireos capably discriminated between neighbours and strangers, showing a strong dear enemy effect. Furthermore, repertoire size had no effect on the strength of neighbour-stranger discrimination across 34 songbirds. These results indicate that large song repertoires have no effect on neighbour-stranger discrimination ability. This conclusion is in agreement with an earlier comparative analysis that investigated the same hypothesis (Weary et al. 1992). Since neighbour-stranger discrimination is valuable to territorial songbirds, it follows that natural selection would select for this ability despite a potential increased difficulty with larger song repertoires.

In Chapter 3, I investigated whether female fertility had an effect on the strength of response to neighbours and strangers in male Song Sparrows. Neighbours are most often the sires of extra-pair offspring in birds (Griffith et al. 2002; Hill et al. 2011; Mennill et al. 2004), therefore increased aggression during periods when neighbours pose a threat to paternity may be a worthwhile investment to ensure parentage. During periods when females are not fertile however, it may benefit males to respond less aggressively to neighbours and conserve energy for other tasks. To test this hypothesis I conducted multiple playback experiments on male Song Sparrows (*Melospiza melodia*) during periods when females were fertile and were not fertile as ascertained by nest monitoring. Male Song Sparrows displayed a dear enemy effect when their female was not fertile but did not display a dear enemy effect when their female was fertile, and thus increased aggression to neighbours. These results suggest that male Song Sparrows respond more aggressively to neighbours during their female's fertile periods as a mate-

guarding tactic to protect their own paternity. My results add to a growing body of work which shows that the dear enemy effect is a flexible phenomenon (Briefer et al., 2008; Courvoisier et al., 2014).

In Chapter 4, I investigated whether the dear enemy effect is observed physiologically in Song Sparrows. I exposed Song Sparrows to either neighbour or stranger playback and collected blood samples to analyze plasma testosterone levels. Testosterone is a steroid hormone that is often implicated in the regulation of aggressive interactions (Wingfield et al. 1990). Song Sparrows exposed to neighbour playback had higher levels of testosterone than Song Sparrows exposed to stranger playback – in contrast to the predictions of the dear enemy effect (e.g. Stoddard et al. 1990). We also observed a dear enemy effect using a qualitative aggression score. This result is puzzling because testosterone levels are known to be associated with aggression in Song Sparrows (Wingfield & Wada, 1989; Wingfield, 1984; Wingfield, 1994) yet Song Sparrows had higher testosterone levels when exposed to a stimulus that elicited lower aggression. One possible explanation is that Song Sparrows use corticosterone instead of testosterone to regulate their responses to conspecific stimuli during incubation in order to avoid any detrimental effects associated with reduced parental care when testosterone is increased. Several studies have found that certain bird species increase corticosterone but decrease testosterone when faced with an aggressive stimulus (Landys et al., 2007; Sorenson et al., 1997; Van Duyse et al., 2004). Corticosterone has been shown to inhibit testosterone production through multiple mechanisms (e.g. Dong et al., 2004; James et al., 2008; McGuire & Bentley, 2010) therefore if corticosterone is

mediating responses to neighbour and stranger stimuli, a decrease in plasma testosterone may be observed. Testosterone does not appear to regulate the dear enemy effect in Song Sparrows and it is clear that more work is needed to understand the role hormones play in dear enemy effect expression.

Future work might extend the work I have presented in this thesis. My second chapter could lead to interesting future research involving repertoire size. It is reasonably clear that repertoire size does not affect neighbour-stranger discrimination but more work could be conducted on the responses of birds to differing repertoire sizes. Researchers could present birds with stimuli consisting of different repertoire sizes to determine if this impacts the aggressive response of birds. If large song repertoires are indicative of male quality, males may respond more aggressively to stimuli consisting of many song types versus stimuli consisting of few song types. Future research stemming from my third chapter could involve a more direct approach to investigating extra-pair copulations. Researchers could investigate whether birds that respond more strongly to neighbour playback during their female's fertile period do indeed have fewer extra-pair offspring in their own nest, thereby validating the idea that increased aggression during fertile periods functions as a mate-guarding tactic. Future research stemming from my fourth chapter could include studies that use a similar design but measure corticosterone levels as well as testosterone levels. It may also be beneficial to capture the neighbour bird and place him out of sight during playback trials in order to ensure that neighbours do not have any confounding effect.

My research elucidates several factors which may drive neighbour-stranger discrimination and the dear enemy effect. Repertoire size does not hinder the dear enemy effect, whereas female fertility status determines male response to neighbours. Testosterone may play a role in dear enemy effect expression however more research is needed to make this clear. My research provides a basis for future research to examine factors that drive or influence the dear enemy effect.

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