

THE FORM AND FUNCTION OF COMPETITIVE TRAITS
IN FEMALE HOUSE WRENS (*TROGLODYTES AEDON*)

By

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ABSTRACT

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Aggressive competition and the signals used during aggressive competition have historically been considered characteristics of male animals produced by sexual selection. Nevertheless, female competitive traits are common across a wide variety of taxa. In most cases, these female traits are understudied. A deeper understanding of how these competitive traits function in female lives is necessary to understand how these traits evolve.

Here I explore two understudied competitive traits in female house wrens (*Troglodytes aedon*), female song and intrasexual aggression. These chapters are connected by three unifying themes: (1) these competitive traits are not merely byproducts of selection on males but instead play functional roles in female lives, (2) although these competitive traits have some similarities to the same traits in males, they are best understood by explicitly considering the contexts in which females compete and the resources that affect female fitness, and (3) (in the case of intrasexual aggression) the same female trait can influence multiple components of female fitness.

In the first half of this dissertation, I examine the form and function of female song and vocalizations that may play a role in female competition. In Chapter 1, I demonstrate that female songs likely play a functional role when defending against conspecific intruders that may usurp the nest cavity, destroy eggs, or threaten future male assistance. In Chapter 2, I examine how particular vocalizations are used during contests with simulated female intruders. I show that a particular call is a signal of aggressive intent. These results collectively suggest that, not only do

these vocalizations have a functional role for female house wrens, but aggressive interactions between rival females may affect female fitness under natural circumstances.

In the second half of this dissertation, I focus explicitly on intrasexual aggression. In Chapter 3, I demonstrate that females are capable of adjusting their aggressive responses in ways that are likely adaptive. I show that females likely value continued access to the nesting cavity more highly than they value direct and indirect benefits offered by males when challenged by simulated female intruders during egg laying. I investigate competition for nesting cavities more explicitly in Chapter 4 by experimentally enhancing the number of competitors searching for cavities. I find that more aggressive females are more likely to win contests over cavities and are more successful at protecting their eggs from intruding conspecifics when competition is intense. In Chapter 5, I look beyond the first half of the nesting cycle to examine the reproductive consequences of female intrasexual aggression throughout the season. In addition to benefits accrued during resource competition, I find that more aggressive females have significantly heavier offspring, a common proxy for offspring quality, even if brood size is experimentally enhanced. More aggressive females are also more likely to raise nestlings to fledging. However, aggressive adults may be less likely to return to the study site in the subsequent year. Taken together, these results suggest that female aggression and female song (which is used in aggressive competition) have important functions and may be under selection in house wrens. While certain benefits (e.g. competition for breeding territories) are likely shared between the sexes, the data suggest that other benefits (e.g. enhanced offspring size) are unique to females. This work highlights the importance of explicitly considering the contexts in which females compete and the resources that influence female fitness when studying intrasexual competition among females.

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INTRODUCTION

Cara A. Krieg

A BRIEF HISTORY OF SEXUAL SELECTION

Extreme weapons, exaggerated ornaments, and bizarrely fantastic displays found in nature have captivated human attention throughout history. From the time of Darwin, biologists have viewed males as the sex most likely to evolve armaments used in conflict with members of the same sex and ornaments used to attract members of the opposite sex (Andersson 1994, Bateman 1948, Darwin 1859, Darwin 1871, Emlen & Oring 1977, Shuster & Wade 2003, Trivers 1972). Theory in the subsequent decades has formalized why sexual selection tends to operate more strongly on males. Variance in access to matings often strongly influences variance in male, but not female, fitness (Shuster & Wade 2003). Thus, selection operates strongly on traits that improve male mating access. Even Darwin recognized that, in a small subset of sex-role reversed species such as pipefish or cassowaries, females are more limited by access to mates than males. Sexual selection has therefore led to the evolution of ornaments and armaments in females instead (Clutton-Brock & Vincent 1991, Darwin 1871, Shuster & Wade 2003). Historically, however, a female's role has been mainly restricted to choosing among suitable males.

Discussions about female ornaments and armaments have historically revolved around one of two perspectives. (1) *Females do not gain the same sexual benefits from ornaments and armaments as males.* Both theory and empirical studies show that females typically do not face a shortage of mating partners (Bateman 1948, Shuster & Wade 2003). Therefore, traits used in

mating competition should be under weaker selection in females. (2) *Female reproductive biology places important constraints on the evolution of exaggerated ornaments and armaments.* Variance in female fitness is often strongly influenced by variance in female fecundity (Shuster & Wade 2003). Furthermore, females in many species invest substantial resources in offspring care following conception. Theory predicts that investing in ornaments and armaments reduces resources that could otherwise be used for reproduction and maternal care, thus lowering female fitness (Chenoweth et al. 2006, Fitzpatrick et al. 1995). For both of these reasons, female ornaments and armaments have historically been considered functionless byproducts of selection operating on males (Amundsen 2000, Lande 1980).

Nevertheless, female ornaments and armaments are quite common. Work in the last decade has highlighted the ever-growing list of species that have these understudied traits and has emphasized the need for alternative approaches for understanding their evolution (Amundsen 2000, Berglund et al. 1993, Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, LeBas 2006, Lyon & Montgomerie 2012, Rosvall 2011a, Rubenstein 2012, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012). Darwin's observation that females do not often compete for matings is correct. However, more recent work suggests the same types of exaggerated traits can evolve in the context of social competition over other critical resources (Clutton-Brock 2007, Clutton-Brock 2009, Stockley & Campbell 2013, Tobias et al. 2012, West-Eberhard 1983). The challenge now is to explain how and why these female traits evolve while explicitly considering the fundamental differences in male and female reproductive biology.

COMPETITIVE TRAITS

Throughout this work, I will refer to the female analogs of male secondary sexual characteristics as “*competitive traits*”. Biologists are currently debating whether and when these female traits should be considered products of sexual selection. By using the term “*competitive traits*”, I intend to remain agnostic with respect to whether sexual selection produces these traits. Broadly, “*competitive traits*” are any trait (e.g. morphology, behavior, etc.) used in competitive interactions with conspecifics for any important resource (Abrams & Matsuda 1994, Cain & Ketterson 2012, West-Eberhard 1983). Thus this term applies to traits used in both mating competition as well as competition for other resources in either sex (Lyon & Montgomerie 2012, West-Eberhard 1979, West-Eberhard 1983). While broad, I believe the term is useful in this instance for several reasons. First, the term itself reflects the context in which these traits are used. Second, the term “*competitive traits*” connects this work with West-Eberhard’s (1979, 1983) social selection theory which is currently becoming popular in the female competition literature (Cain & Ketterson 2013, Lyon & Montgomerie 2012, Tobias et al. 2012). In West-Eberhard’s view, natural, social, and sexual selection can be conceptualized as concentric circles (West-Eberhard 1979, West-Eberhard 1983). This approach is logically and aesthetically pleasing because the same evolutionary force (social selection) can be invoked to explain the same traits in both sexes (e.g. bright colors in male and female birds), even if a more specific form of social selection (i.e. sexual selection) may apply only to one sex. Finally, my aim here is not to determine the specific evolutionary force that produced these traits (e.g. sexual selection, social selection, kin selection, etc.) but rather to examine the costs and benefits that shape them. Whether these female competitive traits could also be considered sexually selected traits depends on the specifics of each case.

FEMALES AND THE SEMANTICS OF SELECTION

At their core, all evolutionary forces (e.g. sexual selection, social selection, kin selection, etc.) can be considered human-imposed division of the broader process of natural selection. These divisions can be very useful. They allow biologists to clearly explain the evolution of traits that would seem to carry high costs (e.g. extreme altruism, fantastically exaggerated traits, etc.). However, if the trait of interest does not separate neatly into one of these divisions, a debate over semantics can ensue that can potentially distract from empirical efforts to understand the trait in question (Clutton-Brock & Huchard 2013, Lyon & Montgomerie 2012, Robinson 2011).

Evolutionary biologists are currently debating whether female competitive traits are products of sexual selection (Rubenstein 2012, Tobias et al. 2012). At the moment, there is no consensus. Some have argued that the historical focus on matings has predisposed the field to overlook female competitive traits and has curiously led to the same trait being considered the product of sexual selection in one sex, yet the product of (non-sexual) natural selection in the other (Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013). These authors have advocated for expanding the definition of sexual selection to include all competition for critical breeding resources. Others have argued this solution will undermine the utility of sexual selection and produce confusion (Shuker 2010). Some think most female competitive traits are better considered under the broader umbrella of social selection (Lyon & Montgomerie 2012, Tobias et al. 2012). Finally, a fringe opinion advocates throwing out sexual selection altogether in favor of a new framework that embraces a diversity of reproductive strategies in males and females (unfortunately labeled “social selection” as well) (Roughgarden & Akçay 2010a, b, but see Clutton-Brock 2010, Carranza 2010, Shuker 2010).

I personally believe this semantic debate can be an unproductive distraction when there is still much important empirical work yet to be done with females (for similar perspective see Cain & Rosvall 2014, Clutton-Brock & Huchard 2013, Robinson 2011, Rubenstein 2012).

Ultimately, male and female phenotypes are the result of natural selection, whatever the specific form. For reasons unique to female reproduction in many species, the outcomes of female competition more often affect fecundity, offspring quality or long-term reproductive success, rather than immediate access to mating partners (Berglund et al. 1993, Clutton-Brock 2009, Rosvall 2011a, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012, but see examples in Berglund et al. 1993, Bro-Jørgensen 2011, Clutton-Brock 2009, Rosvall 2011a). Although true for males as well, it is abundantly clear when looking at females that sexual selection alone is insufficient to explain the existence and specific form of competitive traits. This recent refocus on females is casting new light on the diverse and dynamic ways that natural selection acts through social competition to shape both male and female phenotypes.

THE FORM AND FUNCTION OF COMPETITIVE TRAITS IN FEMALE HOUSE WRENS

In the following chapters, I explore two understudied competitive traits in female house wrens (*Troglodytes aedon*), female song and intrasexual aggression. These chapters are connected by three unifying themes: (1) these competitive traits are not merely byproducts of selection on males but instead play functional roles in female lives, (2) although these competitive traits have some similarities to the same traits in males, they are best understood by explicitly considering the contexts in which females compete and the resources that affect female

fitness, and (3) (in the case of intrasexual aggression) the same female trait can influence multiple components of female fitness.

In the first half of this dissertation, I examine the form and function of female song and vocalizations that may play a role in female competition. In Chapter 1, I demonstrate that female songs likely play a functional role when defending against conspecific intruders that may usurp the nest cavity, destroy eggs, or threaten future male assistance. In Chapter 2, I examine how particular vocalizations are used during contests with simulated female intruders. I show that a particular call is a signal of aggressive intent. These results collectively suggest that, not only do these vocalizations have a functional role for female house wrens, but aggressive interactions between rival females may affect female fitness under natural circumstances.

In the second half of this dissertation, I focus explicitly on intrasexual aggression. In Chapter 3, I demonstrate that females are capable of adjusting their aggressive responses in ways that are likely adaptive. I show that females likely value continued access to the nesting cavity more highly than they value direct and indirect benefits offered by males when challenged by simulated female intruders during egg laying. I investigate competition for nesting cavities more explicitly in Chapter 4 by experimentally enhancing the number of competitors searching for cavities. I find that more aggressive females are more likely to win contests over cavities and are more successful at protecting their eggs from intruding conspecifics when competition is intense. In Chapter 5, I look beyond the first half of the nesting cycle to examine the reproductive consequences of female intrasexual aggression throughout the season. In addition to benefits accrued during resource competition, I find that more aggressive females have significantly heavier offspring, a common proxy for offspring quality, even if brood size is experimentally enhanced. More aggressive females are also more likely to raise nestlings to fledging. However,

aggressive adults may be less likely to return to the study site in the subsequent year. Taken together, these results suggest that female aggression and female song (which is used in aggressive competition) have important functions and may be under selection in house wrens. While certain benefits (e.g. competition for breeding territories) are likely shared between the sexes, the data suggest that other benefits (e.g. enhanced offspring size) are unique to females. This work highlights the importance of explicitly considering the contexts in which females compete and the resources that influence female fitness when studying intrasexual competition among females.

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CHAPTER ONE

Not just for males: female house wrens use song against
male and female rivals in a temperate zone population

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ABSTRACT

Birdsong in temperate zone passerines is a trait under sexual selection in males. Female song is still thought to be rare in this group. Here we show that female song is common in a temperate zone population of house wrens, *Troglodytes aedon*, and we provide evidence for its functional role in defending against male and female conspecifics. We observed that females sang most frequently at the onset of egg laying, with song becoming less common as incubation approached. Thus, females sang most during the time when eggs were left unguarded and susceptible to conspecific attack. We also conducted playback experiments to test whether conspecific stimuli would induce female song in focal individuals. Playback from both male and female conspecifics elicited strong song responses from resident females, who often vocalized independently from their partners. However, females were more physically aggressive towards female songs than male songs. Finally, females that sang more during these simulated conspecific intrusions ultimately lost fewer eggs to house wren ovicide. These results suggest that female house wren song may have evolved, at least in part, for use in intra and intersexual competition. These results also highlight how investigating these traditionally male behaviors in female animals can lead to key insights regarding the evolution of sexual dimorphism.

INTRODUCTION

Analyses of birdsong are a cornerstone in the sexual selection literature. Birdsong is a classic example of a male trait under both intrasexual selection for territory defense and intersexual selection for mate attraction (Catchpole & Slater 2008). In contrast to male song, female song is much less common in temperate zone breeding species (Langmore 1998; Morton 1996; Slater & Mann 2004). The earliest and most thoroughly studied birdsong systems come from temperate zone species where females are largely songless (Catchpole & Slater 2008; Slater & Mann 2004). In contrast to their temperate zone counterparts, many tropical females are prolific singers, performing coordinated vocal duets with males that function to defend territorial resources, guard their mate against intruding females, solicit copulations from their mates, and/or coordinate pair and reproductive behavior (for review see Farabaugh 1982; Hall 2004; Langmore 1998). Because of the history of birdsong studies, songless temperate zone females have been seen as the archetypal condition whereas tropical singers have been seen as a derived state.

Recent phylogenetic work has rewritten this narrative. A survey of 34 songbird families conducted by Odom et al. (2014) reconstructed female song as an ancestral state for all oscine passerines. This large-scale pattern is consistent with new findings for several avian families (Icteridae: Odom et al. 2009; Price et al. 2009; Muscicapidae and Fringillidae: Garamszegi, Pavlova et al. 2007). These studies found that female song is an ancestral condition that has been repeatedly lost as species adopt a temperate zone or migratory breeding pattern. To understand the full picture of birdsong evolution, it is important to understand why female song was lost or maintained in temperate zone lineages, not merely why tropical females sing (Price 2015; Riebel et al. 2005).

Two major hurdles stand in the way of this goal. The first is the shortage of systematic studies of female song in temperate zone breeding species (Garamszegi et al. 2007). Anecdotes of temperate zone female singing have historically been dismissed as the byproduct of abnormally high steroid hormone levels (Byers & King 2000; Gilbert & Carroll 1999; Nice 1943; Ogden et al. 2003; Thorpe 1964), although this hypothesis is rarely supported by hormonal sampling. This lack of hormonal evidence makes it difficult to determine whether this behavior is truly a biological abnormality or merely an unexpected natural phenomenon. The second major hurdle is the lack of data on the function of temperate zone female song. A number of temperate zone species are known to possess female song. This behavior occurs regularly in several species (alpine accentor, *Prunella collaris*, European starling, *Sturnus vulgaris*, northern cardinal, *Cardinalis cardinalis*, red-winged blackbird, *Agelaius phoeniceus*), occasionally in some (black-capped chickadee, *Poecile atricapillus*, black-headed grosbeak, *Pheucticus melanocephalus*, dunnock, *Prunella modularis*, European robin, *Erithacus rubecula*, song sparrow, *Melospiza melodia*, tree swallow, *Tachycineta bicolor*, white-crowned sparrow, *Zonotrichia leucophrys*, white-throated sparrow (white-striped morph), *Zonotrichia albicollis*, yellow warbler, *Setophaga petechial*), and rarely in several others (Baltimore oriole, *Icterus galbula*, chestnut-sided warbler, *Setophaga pensylvanica*, common yellowthroat, *Geothlypis trichas*, hooded warbler, *Setophaga citrina*, indigo bunting, *Passerina cyanea*, rufous-sided towhee, *Pipilo erythrophthalmus*, Wilson's warbler, *Cardellina pusilla*) (see Langmore 1998, for partial review; additional species: Beletsky 1982; Byers & King 2000; Gilbert & Carroll 1999; Hahn et al. 2013; Lowther & Falls 1968; Nolan 1958; Ogden et al. 2003; Sharman et al. 1994; Taff et al. 2012). For most of these species, however, the function of song remains unknown.

Here we examine several possible functions of female song in a temperate zone population of house wrens, *Troglodytes aedon*. Female house wren song has only been reported in one previous temperate zone population during a larger study on male vocal behavior (Johnson & Kermott 1990b). These authors concluded that female song is directed at the female's mate, functioning to redirect male attention back to the female during periods of coordinated behavior. Like those authors, we found that female singing is common in our population. However, we also observed that it is frequently performed in the absence of males. Based on this new observation, we examined additional possible functions of song in female house wrens.

Both male and female house wrens compete to obtain and defend valuable breeding resources. House wrens are small (10–12 g), facultatively polygynous migratory songbirds that nest in secondary cavities (Kendeigh 1941). Both males and females compete separately for access to limited nest cavities (Johnson & Kermott 1990a; Johnson & Searcy 1996). Each year we observe several instances of male–male and female–female replacements on territories. Males frequently advertise for secondary mates once the primary female begins incubating (Johnson & Kermott 1991a), although 5–45% of males may be polygynous depending on the population and year (Drilling & Thompson 1991; Johnson & Kermott 1991a, 1991b; Kendeigh 1941). House wrens also engage in ovicide. Prior to laying their own eggs, both male and female house wrens will puncture and toss any undefended songbird eggs they find in other nests nearby (Belles-Isles & Picman 1986). Ovicide directed at other wrens also occurs in conjunction with attempted or successful territory takeovers, resulting in the destruction of 4–15% of the eggs in our population each year (Krieg n.d.).

We focus on three possible functions of female house wren song that parallel the functions of song in male songbirds (Slater & Mann 2004). All female song data for the present study were collected in the vicinity of active nests (≤ 30 m). Therefore, we limited the hypotheses to those we could address by measuring song near the nest. We asked whether female house wren song works to (1) coordinate pair behavior, (2) solicit copulations or (3) defend against conspecific threats to eggs and paternal care. Each of these hypotheses predicts that female singing should peak at different times of the female nesting cycle (see Figure 1.1) and that females should respond to simulated conspecifics in different ways. If females sing to coordinate pair behaviour, songs should peak prior to egg laying and during the nestling period when house wren pair behaviour is highly coordinated (Figure 1.1a) (Johnson & Kermott 1990b). Females should not sing in response to simulated conspecifics under this hypothesis. If instead females sing to solicit copulations, songs should peak during the female's fertile period that starts several days prior to egg laying and lasts through the day of the penultimate egg (Figure 1.1b) (Johnson & Kermott 1989). Females may only sing in response to simulated male conspecifics if songs are designed to solicit extra-pair copulation or fail to sing if solicitation songs are directed only at her social mate. Finally, if females sing to ward off conspecific threats, songs should peak when conspecifics pose the biggest risk to female interests (Figure 1.1c). Conspecific intruders are the biggest threat during the egg-laying period. Female house wrens do not begin brooding their eggs until the clutch is complete, so eggs face the greatest threat of ovicide prior to incubation when their eggs are left unguarded for long stretches of time. Nestboxes are also likely easiest to usurp during this period for the same reason. Furthermore, if a secondary female begins her clutch around the same time as a primary female, their nestling periods will overlap, and the male will be forced to split his attention (see Appendix A). Therefore, a primary female should

work particularly hard to repel prospecting females at the beginning of her egg-laying period (Slagsvold & Lifjeld 1994). This hypothesis predicts that females will sing strongly in response to simulated conspecifics, particularly female conspecifics. We noted all instances of spontaneous female song observed during routine visits across the nesting cycle and conducted a playback experiment during the beginning of egg laying to determine which hypothesis best explained the patterns observed in female house wren song.

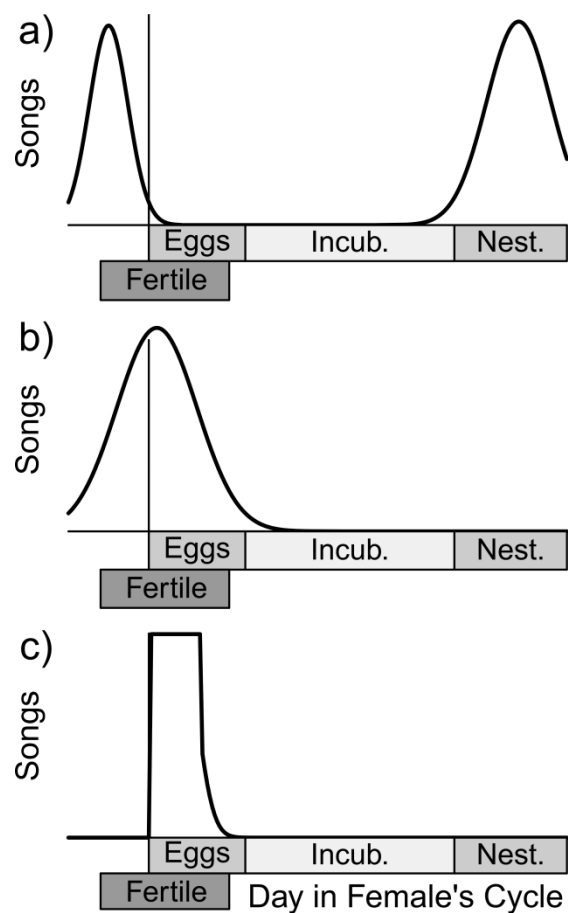


Figure 1.1: Predictions for the timing of female singing if song is used to a) coordinate pair behavior, b) solicit copulations, or c) defend against conspecific threats to eggs and paternal care for each phase of the female's nesting cycle (fertile period, egg-laying period, incubation period, and nestling period).

METHODS

Study Site

This study was conducted at the Lux Arbor Reserve, Kellogg Biological Station in Barry County, MI, U.S.A. (42°29'N, 85°28'W). This reserve is a 529 ha protected area containing grasslands, wetlands, forest, and fragmented agricultural fields. Prior to the return of the house wrens from migration in April, we set up wooden nestboxes near forest edges on 59 territories. Each territory contained a cluster of three boxes situated 5–10 m apart, and each cluster was roughly 100 m from its neighbor, allowing one male to easily defend a single cluster but making it difficult to defend more than one. This population had been established for 15 years prior to the present study (Dubois & Getty 2003) and attracts roughly 100 adults each year that we colour-band for individual identification.

Female Song

We define female song as a stereotyped female vocalization lasting longer than 1 s. Female song in this species ranges from a collection of buzz-like broadband syllables to songs that are nearly indistinguishable from male songs (Appendix A, Figure 2.7). These female songs are distinct from both the chattering calls females give when the nest is disturbed by predators and the high-pitched calls given sometimes during courtship (C. A. Krieg, personal observation). Recordings from Appendix A, Figure 2.7 are available in the online song repository <http://www.xeno-canto.org>.

We identified female song types by either directly observing a colour-banded female singing or by matching the structure of the song to a female song previously verified in such a fashion. Visual confirmation was possible during the majority of observations. Nearly all female songs are structurally distinct from male song and easy to distinguish by ear. For the one female

that sang a very male-like song, we were stricter about attributing songs to the female. Each song was either given when the female was directly visible or given from a location where we saw the female disappear, during which time her mate was clearly present in a different location. We never observed a third house wren on this territory.

Spontaneous Female Song

During the summers of 2012 and 2013, we checked nestboxes for activity every 1–4 days and noted spontaneous female song any time it occurred. We define spontaneous song as a song that was not provoked by experimental house wren playback. We measured spontaneous song in three different nest visit scenarios (standard box checks, nestling measurements, pre-playback period of trials) using three criteria that allowed us to compare across these contexts. First, we limited our data set to songs that occurred during the first 5 min spent within 30 m of the nest that day. Second, all three contexts involved a brief approach to the nest and thus a similar level of disturbance. Third, for each day, no spontaneous songs were measured after house wren playback was used or after nestlings were temporarily removed. During standard box checks, an observer listened for all vocal activity as they approached the territory and took notes on the status of the nest, the vocal behavior of the male and female resident, the closest distance each bird approached and the identity of each bird. During nestlings measurements, the same data were taken and nestling were measured at five points in 2012 (days 2, 4, 6, 8 and 10 after hatching) and at four points in 2013 (days 4, 6, 8 and 10). The playback experiment is explained in further detail below (see Playback Experiment).

It is possible that some females sang in direct response to the human observer near the nest. However, house wrens at our study site are regularly exposed to humans throughout the breeding season. When agitated, house wrens typically respond with chattering alarm calls (C. A.

Krieg, personal observation). They appear to habituate quickly to human presence when observers retreat some distance from the nest and remain quiet, allowing observers to view typical courtship, nest building, incubation, and feeding behaviors.

We used the date that the first egg was laid by each female (defined as day 0) to determine the day in the female nesting cycle on which these spontaneous songs occurred. We define the ‘nesting cycle’ as the period between nest building and fledging of nestlings. For the purposes of this study, the nesting cycle was limited to 5 days prior to egg laying (day -5) until 28 days after the first egg was laid (day 28). The location of most females was not known prior to day -5 and nests were not visited after day 28 to prevent startling nestlings into early fledging. Cycle days do not perfectly correspond to calendar date within the breeding season as females settle asynchronously and typically have two clutches per season. Each time a new clutch was initiated, we considered this a new nesting cycle.

Playback Experiment

To evaluate song responses to different types of simulated intrusions, we tested each female with playback from a female house wren, a male house wren, and a heterospecific control (described below). All subsequent references to ‘trials’ refer to one of these tests. For each trial, a speaker was attached to a tripod 1.5 m off the ground and positioned within 2 m of the active nestbox. For male and female playback trials, we placed a painted wooden carving of a house wren on top of the tripod to give birds a potential target for aggression. House wrens are sexually monomorphic in both size and coloration, so we used the same model for both male and female trials. Each playback trial consisted of a 2 min set-up period during which no data were recorded, a 5 min silent pre-playback period, a 5 min playback period, and a 5 min silent post-playback period. An observer (C.A.K.) with a Marantz PMD 660 solid-state digital recorder attached to a

Sennheiser ME62 omnidirectional microphone and a Mineroff parabolic reflector recorded all vocal responses from the focal pair and narrated any physical attacks females directed towards the playback set-up. The sex of responding birds was easy to determine using acoustic characteristics and leg band colors.

All females were tested with only one of the three trial types each day until all three trial types were completed. We conducted tests between sunrise and 1230 hours Eastern Standard Time within the first 4 days of the egg-laying period, with trial order assigned randomly. We tested most birds on 3 consecutive days, but some birds experienced a 1 day gap because of weather or time constraints. In 2012, we tested 53 females, and in 2013, we tested 56 females, 17 of which were also tested in 2012. Some birds received an additional set of trials, but we only included the first set in this analysis. Some birds did not receive all trial types as a result of uncontrolled nest destruction (12.8% of trial sets).

We constructed each playback stimulus from a loop of six different high-quality songs given by the same individual. Each song was separated by 5 s of silence to approximate natural singing rates. In the first year of playback studies (2012), we constructed six unique female stimuli and six unique male stimuli each from recordings of a single known individual from 2011. In the following year (2013), we expanded the stimulus set to include 16 female recordings and 14 male recordings made from known individuals from both 2011 and 2012. The six unique control stimuli used in both years were each composed of six high-quality songs from six different cup-nesting species recorded at the study site in 2011 (American robin, *Turdus migratorius*, grey catbird, *Dumetella carolinensis*, eastern towhee, *Pipilo erythrophthalmus*, song sparrow, *Melospiza melodia*, chipping sparrow, *Spizella passerina*, field sparrow, *Spizella pusilla*). We filtered out all noise below 1 kHz and normalized all songs to a peak amplitude of

1.4 kU using Raven Pro v.1.4 (Bioacoustics Research Program 2011). Birds were never tested with their own song, the song of their mate, song stimuli that they were tested with in a previous year or the song of neighbors that shared a territory boundary. The results presented here were qualitatively similar for 2012 and 2013 and are therefore pooled for all subsequent analyses.

House Wren Ovicide

Throughout the season, we tracked the status of the nestbox and noted the cause of any egg destruction. Wren ovicide leaves telltale signs. We classified eggs as destroyed by ovicide if eggs were (1) found cracked but unconsumed, (2) found with beak-sized punctures, (3) found unconsumed on the ground in front of the box, or (4) missing on the day that a new wren moved on to the territory. We classified eggs as destroyed by predators if (1) shells were found in the nest, (2) the nest or nestbox was clearly disturbed or damaged or (3) a nest predator was found living in the box. We classified the cause of destruction as ‘unknown’ if none of these criteria applied.

Statistical Analysis

We used a chi-square goodness-of-fit test to determine whether visits with spontaneous female song occurred more often on particular days in the female’s nesting cycle than expected by chance, given our observational effort. We visited nests more frequently during certain periods of the female’s cycle (e.g. egg laying) than others (e.g. incubation). We used the number of nest visits we made on each day in the cycle to calculate the number of songs expected based on an equal probability of female song occurring on each visit. We restricted our chi-square analysis to the period between -5 days and +8 days, because including the low numbers of expected songs during the incubation and nestling periods violated the assumptions of the chi-square test (day of the first egg was set at 0 days, and females laid 5–8 eggs per clutch). The

three hypotheses about the function of female song differ most in their predictions during this period. A day with a chi-square value greater than 3 was considered to contribute strongly to the lack of fit with the random expectation.

We then used a negative binomial generalized linear mixed model with a log link and Tukey post hoc contrasts to test whether female song occurred more often during some stages of the playback trials than others. The dependent variable was the number of female songs, the independent variable was playback stage, and female identity was included as a random effect.

Finally, we used a Wilcoxon signed-ranks test to determine whether females initiated more vocalization bouts during and after playback for both the control, female and male trials. We applied a Holm familywise error rate correction to the *P* values from these three tests to correct for multiple comparisons (Holm 1979). Females in our experiment sang (1) solos, (2) songs that were followed by male songs upon their conclusion, (3) songs that were given after male songs concluded, as well as (4) songs that overlapped with male songs. When female songs occurred 3 s or less from the start of the nearest male partner song, we considered these vocalizations initiated by the sex that sang first, regardless of whether these vocalizations actually overlapped. All other songs were considered female solos. Vocalization bouts initiated by females as well as female solos were considered female-initiated vocalizations. When two house wren songs start within 3 s of one another, these vocalizations either overlap or occur in close sequence. Reducing this cutoff to 1 s or expanding it to 6 s does not change the significance of our results. These distinctions allowed us to determine whether females were sometimes operating independently when deciding to vocalize, or whether they were exclusively following their mate's lead. All tests are two tailed. We performed all statistical tests with R version 3.0.2 (R Core Team, 2013; package 'glmmADMB': Fournier et al. 2012; Skaug et al.

2015; package ‘multcomp’: Hothorn et al. 2008; package ‘car’: Fox & Weisberg 2011; package ‘MuMIn’: Barton 2015).

Ethical Note

This research was approved by the Michigan State University’s Institutional Animal Care and Use (IACUC) panel, followed the ASAB/ABS Guidelines for the use of animals in research, and followed appropriate federal and state permits (Michigan State University IACUC number 04/11/076-00; U.S. Fish and Wildlife Service Federal Banding Permit number 23587; Michigan Department of Natural Resources Michigan Scientific Collectors Permit number SC 1432). Female reactions to recorded song were similar to their reactions to ambient natural song, and they returned to pretrial behavior within 30 min. No birds abandoned territories following our tests. Females were either trapped on the nest during incubation or in mist nets that were monitored continuously using procedures deemed appropriate by Michigan State University’s IACUC panel, the U.S. Fish and Wildlife Service and the Michigan Department of Natural Resources. No birds suffered adverse effects during brief capture and measurement.

RESULTS

Prevalence of Female Song

Female song is a common phenomenon in our population of house wrens. Out of the 108 adult females that were present during 2012 and/or 2013, 72 females (66.7% of the study population) were observed singing in at least one of the three contexts examined. Thirty-one females were observed singing spontaneous female song according to the methods outlined in this study (28.7% of studied females). In response to playback, 60 of the 92 tested females sang during at least one trial (65.2% tested females). An additional 17 birds not included in the

analyses above were observed singing either spontaneous song during longer observations on focal territories (15 birds) or provoked song during mist net attempts using house wren playback (2 birds) (see Appendix B). Like most temperate zone birds, however, male house wrens still sang more frequently. All males sang at some point, and we heard male songs during 33% of nest visits compared to 2% of nest visits for female songs.

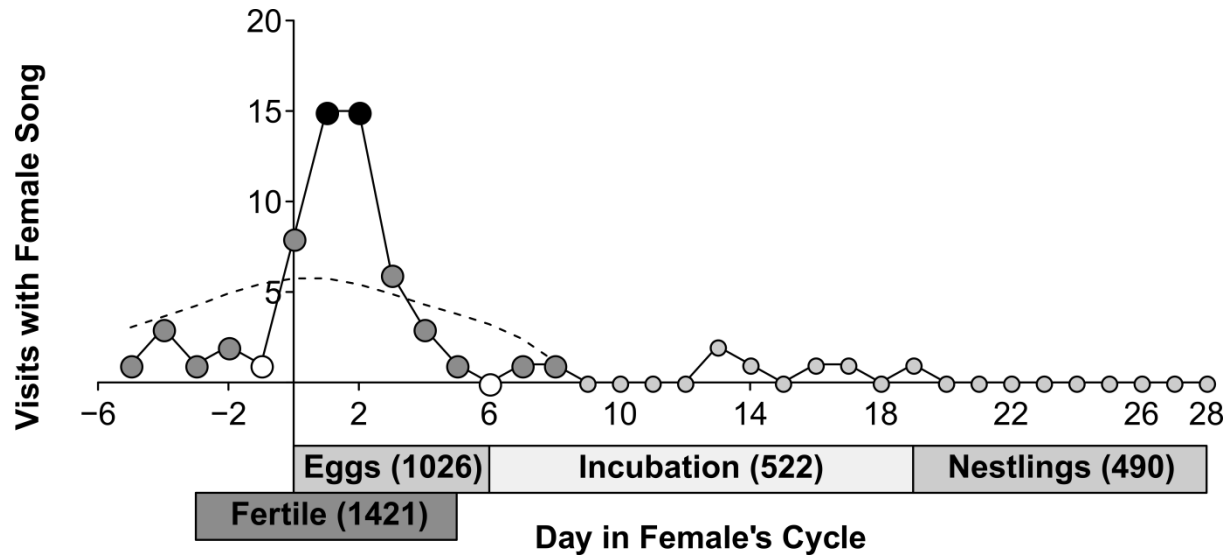


Figure 1.2: Number of nest visits with spontaneous female song observed (solid line) and expected (dashed line), if song is equally probable on each nest visit, across the nesting cycle. Black dots represent days when songs were more common and white dots represent days when songs were less common than expected ($\chi^2 > 3$). Light grey dots after day 8 were not included in the chi-square analysis because of low sampling effort and expected values. Numbers in parentheses indicate the number of nest visits made during each stage.

Spontaneous Female Song

Females sang during significantly more visits on particular days than would be expected by chance (chi-square test: $\chi^2_{13} = 48.54$, $P < 0.0001$; Figure 1.2). Most visits with spontaneous female song occurred at the beginning of the egg-laying period. Visits with spontaneous female song were rare on days prior to egg laying and then spiked dramatically by the day the second

egg was laid (cycle day 1). By the end of the egg-laying period, song declined once again to low levels that were maintained for the remainder of the nesting cycle.

The timing of this increase in female song did not appear to be merely a result of increased vocal vigilance on days following playback of conspecific song. During the peak of singing (day 0–3), we observed 24 visits with spontaneous song before any house wren playback was used on the territory and 20 visits with spontaneous song after house wren playback had been used on a previous day.

The pattern of singing across the nesting cycle was consistent with both the copulation–solicitation hypothesis and the defense against conspecifics hypothesis (see Figure 1.1b, c). Note, however, that songs were significantly less common than expected during visits on the day before the first egg was laid, which was well within the female’s fertile period. The defense against conspecifics hypothesis is supported further by the results from our playback experiment.

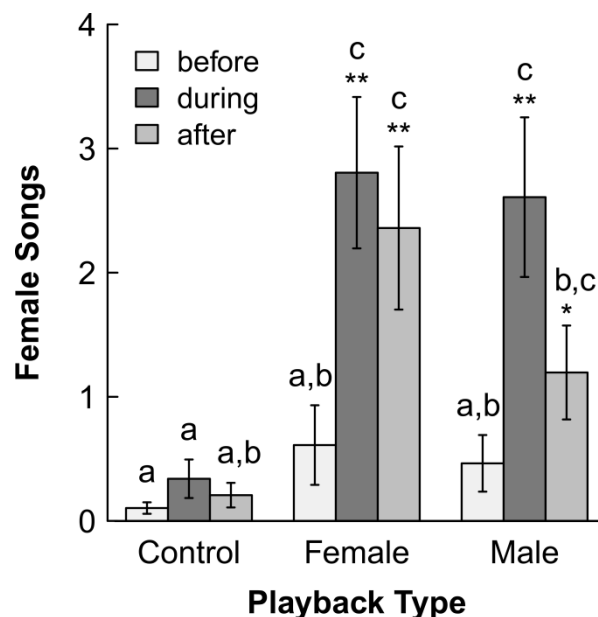


Figure 1.3: Mean $\pm$ SE number of female house wren songs recorded during each 5 minute playback stage (before, during, and after playback) in response to heterospecific (control) songs, female conspecific songs, and male conspecific songs (* $P < 0.05$; ** $P < 0.01$). Letters represent Tukey’s post hoc comparisons.

Female Response to Playback

Playback of conspecific song prompted dramatic increases in female house wren singing. Females sang significantly more during and after both female and male playback compared to the playback stage of control trials (negative binomial generalized linear mixed model: during female playback: $Z = 6.77$, $P < 0.001$; post female playback: $Z = 6.07$, $P < 0.001$; during male playback: $Z = 6.04$, $P < 0.001$; post male playback: $Z = 3.07$, $P = 0.049$; Figure 1.3). The majority of female songs occurred following conspecific playback, and females that sang gave more songs on average following conspecific playback than during control playback or silence (Table 1.1). Furthermore, females were not exclusively following their partner's vocal response when engaging with conspecifics (Figure 1.4). Females initiated the majority of songs during and after both female playback (Wilcoxon signed-ranks test: $V = 857$, $N = 108$, corrected $P < 0.0001$) and male playback ($V = 662.5$, $N = 97$, corrected $P = 0.005$). However, during and after control playback, the number of songs that females initiated independently (female-initiated) and the number songs that females gave immediately following songs of their mates (male-initiated) did not differ ($V = 53$, $N = 106$, corrected $P = 0.266$). Previous house wren playback did not significantly change female singing responses to subsequent house wren trials. Including whether the trial was performed before or after a previous house wren trial as a model coefficient did not improve the model fit or alter the significance of our results.

While females responded to both male and female playback with singing, female songs provoked the strongest physical response. A total of 16 different females (17.4% of tested females) attacked the playback by swooping down within inches of the model or speaker, sometimes making direct physical contact. Female playback received the majority of these attacks (94.7%) (Table 1.1).

Table 1.1: Number of attacks and number of songs sung by female house wrens during each stage of playback trials

Trial Type	During and after playback			Pre-playback
	Female ^a	Male ^b	Control ^c	All
Number of attacks	36	2	0	0
Attacks per attacking female (mean $\pm$ SE)	2.4 $\pm$ 0.57	1 $\pm$ 0	-	-
Number of songs	601	369	58	122
Songs per singing female (mean $\pm$ SE)	13.98 $\pm$ 2.36	8.39 $\pm$ 1.94	4.24 $\pm$ 1.61	3.94 $\pm$ 1.17

^a $N = 108$ trials, 91 individuals

^b $N = 97$ trials, 82 individuals

^c $N = 106$ trials, 89 individuals

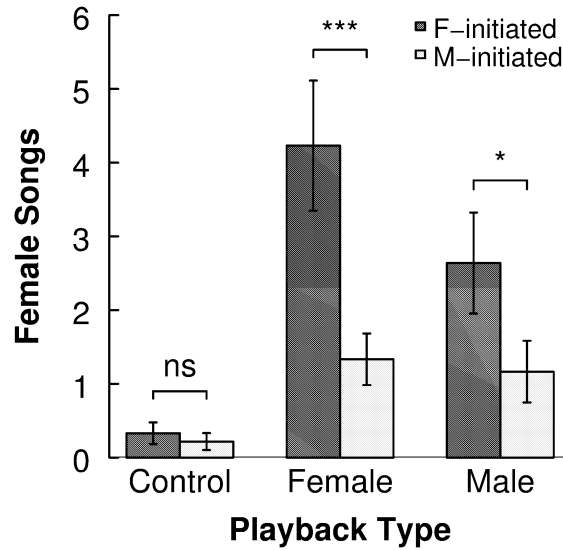


Figure 1.4: Mean $\pm$ SE number of songs during and after playback that were initiated by female (F-initiated) and male (M-initiated) house wrens in response to heterospecific (control) songs, female conspecific songs, and male conspecific songs (* $P < 0.05$; ** $P < 0.01$).

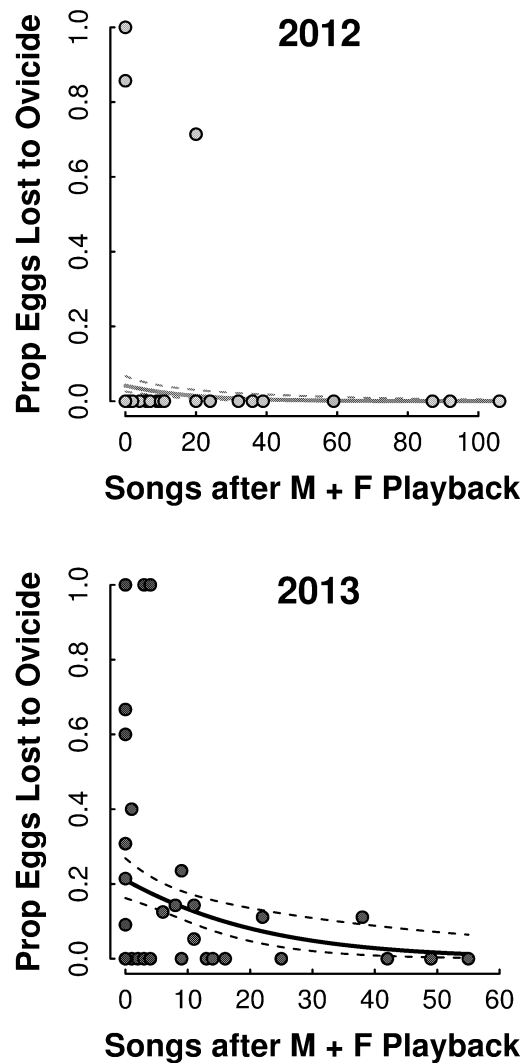


Figure 1.5: Relationship between the proportion of eggs that female house wrens lost to ovicide and the number of songs females sang during and after playback of male and female conspecific song in 2012 and 2013 (binomial generalized linear model: model $R^2 = 0.25$, songs $P < 0.001$). Lines represent predicted values for each year in the model $\pm$ 95% confidence intervals.

Reproductive Consequence of Female Song

To further evaluate the defense against conspecifics hypothesis, we asked whether females that sang more during simulated intrusions were better at defending their eggs (binomial generalized linear model, fixed effects: songs + year). We found that females that sang more songs during the playback and post-playback period of the male and female trials lost

significantly fewer eggs to house wren ovicide (model $R^2 = 0.25$, songs: $Z = -3.34$, $P = 0.0008$; Figure 1.5). The rate of ovicide was significantly higher in 2013 than in 2012 (year: $Z = 6.3$, $P < 0.0001$). In 2013 we carried out a different experiment that briefly increased the number of floating house wrens. The population was also larger in 2013 (27% more females). While these factors probably increased ovicide rates in 2013, the relationship between song and ovicide was qualitatively similar in 2012 (see Figure 1.5). Although female song may also prevent secondary females from settling, rates of polygyny were too low in our population to evaluate this dimension of the defense against conspecifics hypothesis.

DISCUSSION

Here we present evidence of temperate zone females using their songs in a similar fashion to temperate zone males. Female house wren song appears to play a role in defending against reproductive threats from both male and female rivals. Females sang most frequently at the onset of egg laying, during which conspecific intruders are the biggest threat to vulnerable eggs and future paternal care. This behavior decreased dramatically as incubation approached and remained at a low level for the rest of the nesting cycle. Furthermore, simulating conspecific intruders by playing male or female playback elicited strong song responses from female house wrens. These females were not merely following the defense efforts of males. Females initiated the majority of their song bouts following conspecific playback, in contrast to the control condition, in which they were equally likely to follow the male's lead. Finally, this female behavior had measurable reproductive consequences. Females that gave more songs in response to simulated male and female intruders lost significantly fewer eggs to house wren ovicide.

While females were equally vocal to male and female stimuli, females were more aggressive towards female stimuli. Nearly all physical attacks were directed exclusively at female playback songs. This suggests that females may have perceived other females as a greater threat than other males. During the egg-laying period, both male and female residents benefit from driving away other males that may usurp territories and destroy eggs. Female intruders pose a unique threat to resident females because they will not only destroy eggs but may also settle as a secondary female. Secondary females are the biggest threat when they begin their clutch around the same time as a primary female (Slagsvold & Lifjeld 1994). In this scenario, the period of nestling provisioning will overlap for both females, and males must split their attention (see Appendix A). While primary females may also benefit from preventing female settlement at the beginning of incubation, the time constraints of incubation probably limit their defensive efforts.

In the tropics, where both sexes generally maintain their territories year-round, females frequently use song to defend territories (for examples see Brunton et al. 2008; Cooney & Cockburn 1995; Farabaugh 1982; Hall 2004; Illes 2015; Illes & Yunes-Jimenez 2009; Levin 1996). In temperate zone species, female song also appears to play a role in defense (for examples see Arcese et al. 1988; Baptista et al. 1993; Beletsky 1983; Hobson & Sealy 1990; Langmore 1998, 2000; Sandell & Smith 1997). Although vocal displays during conspecific conflicts by temperate zone males, tropical females, and temperate zone females take the same form, the functions of these displays can be very different. While males certainly use song to defend nonsexual resources, this behavior is frequently studied in the context of sexual selection. Temperate zone males singing to defend temporary breeding territories accrue benefits typically not sought by temperate zone females (i.e. defense of a resource to attract mates and prevention of extrapair fertilizations by neighboring males; Catchpole & Slater 2008; Morton 1996). In

contrast, tropical birds of both sexes often defend year-round territories and form long-term pair bonds, making territories and mates particularly valuable resources to both males and females (Farabaugh 1982; Morton 1996; Slater & Mann 2004). In one sense, female house wrens behave in a fashion similar to males when singing to protect eggs, nesting cavities and/or paternal investment from conspecific threats. In another sense, this behavior differs in important ways.

Like female house wrens, other females in the wren family (Troglodytidae) use their songs during inter and intrasexual competition. The wren family contains a wide range of female singing styles, culminating in species that perform complex, antiphonal duets (Mann et al. 2009). In temperate zone species, female song is thought to be absent (marsh wren, *Cistothorus palustris*: Brenowitz et al. 1994; Carolina wren, *Thryothorus ludovicianus*: Nealen & Perkel 2000) or its function is unknown (canyon wren, *Cartherpes mexicanus*: Benedict et al. 2013). In tropical species, where female song function has been investigated, it appears to play a role in inter and/or intrasexual territory defense (buff-breasted wren, *Cantorchilus leucotis*: Gill et al. 2007; bay wren, *Cantorchilus nigricapillus*: Levin 1996; black-bellied wren, *Pheugopedius fasciatoventris*: Logue & Gammon 2004; rufous-and-white wren, *Thryophilus rufalbus*: Mennill 2006; happy wren, *Pheugopedius felix*: Templeton et al. 2011; but see banded wren, *Thryophilus pleurostictus*: Hall et al. 2015). With the wide range of singing styles and natural histories found in Troglodytidae, this family may be a particularly useful model for investigating the evolution of female song.

Although the results here suggest that females use their song to defend nest areas from conspecifics, this may not be the only function of this behavior. Female house wren song occurs occasionally throughout the entire nesting cycle, even during periods where conspecifics are not significant threats to reproductive activities. Females may also use song differently at territory

edges or off their territories, for instance, to defend territory boundaries or seek extrapair copulations. Without more systematic measurement of female vocal behavior throughout a female's range, we are unable to offer an exhaustive determination of all the possible functions of female song. Instead, we offer one previously unknown function of female song in a species where female song has received little attention. Female house wrens may sing both to communicate with their mates, as suggested by Johnson and Kermott (1990b), as well as to defend eggs, nesting cavities, and/or paternal investment from conspecifics, as our results suggest. Like male song, female house wren song probably plays a role in both intra and intersexual communication.

The nature of female house wren vocal behavior highlights challenges facing researchers studying other temperate zone singers. Although the majority of the females in our population were observed singing, this behaviour has only been documented once before (Johnson & Kermott 1990b). Why has this behavior been largely overlooked? First, house wrens are sexually monomorphic. Because song is only expected to occur in temperate zone males, field biologists may be likely to assume that a singing bird of unknown sex is a male (Langmore 1998). This appears to have been the case in black-capped chickadees, another monomorphic species (Hahn et al. 2013). Second, the majority of female house wren song is vocally distinct from male house wren song. Unless one first clearly observes a female making this unique sound, it would be extremely easy to dismiss female song as an unknown vocalization from a different species (see Nolan 1958, for a similar case). Third, female house wren song is common during a specific window of the breeding season and is used in a specific social context. If observers are not in the right place at the right time, it is easy to miss entirely. Male house wrens sing most when attempting to attract females to their territories, and they dramatically reduce both the quantity

and amplitude of singing during egg laying (Johnson & Kermott 1991b). Biologists focused only on male house wren vocal behavior could easily miss the peak of female singing. If other female singers are using their songs differently than males, this may be difficult to detect without a specific focus on females. To understand the broad patterns of female song evolution, we need to understand the selective forces favoring the maintenance of these vocalizations. To accomplish this, however, we may first need to re-evaluate which temperate zone species truly lack female singers and which hold unexpected surprises.

Predicting which species are likely to possess female song requires a more detailed examination of the relevant natural history characteristics. When females compete for valuable breeding resources, selection should favor aggressiveness as well as signals used in aggressive encounters in a process analogous to sexual selection on males (Clutton-Brock 2009; Clutton-Brock & Huchard 2013; Rosvall 2011; Tobias et al. 2012; West-Eberhard 1983). Female house wrens lack many of the characteristics currently associated with female song (tropical breeding, year-round territoriality, social monogamy, sedentary behavior) (Odom et al. 2015; Price 2009; Price et al. 2009; Slater & Mann 2004). However, as facultatively polygynous secondary cavity nesters, they are in direct competition with other females for limited nesting cavities and valuable paternal care. Polygynous females are expected to face high levels of intrasexual competition (Slagsvold & Lifjeld 1994). Many temperate zone species with female song also demonstrate occasional polygyny (for partial review see Langmore 1998, 2000; additional species: Carey & Nolan 1975; Clemmons 1994; Flood 1985; Powell & Jones 1978; Quinney 1983). Rival females may pose an additional threat in secondary cavity-nesting species, which typically face intense competition for suitable nesting sites (von Haartman 1957). Under natural conditions, only a portion of house wren males have access to these supplemental nests (Czapka

& Johnson 2000; Johnson & Kermott 1991a). Ovicide by nearby females probably further reduces the number of viable cavities. Intriguing, European starlings, which demonstrate frequent female singing, are also facultatively polygynous cavity nesters that elevate singing rates when defending nestboxes from other females (Pavlova et al. 2007; Sandell & Smith 1997). These additional dimensions of female competition in house wrens may explain why a species with multiple characteristics associated with the loss of female singing still possess functional female song.

Most studies to date have investigated factors that differ dramatically between temperate zone and tropical species when considering the evolution of female song, such as migratory behavior or broad natural history changes associated with tropical environments (Odom et al. 2015; Price 2009; Price et al. 2009; Slater & Mann 2004). Here we show that female song also has a function for certain migratory temperate zone species. Our results suggest that additional factors more intimately related to the possible function of female song, for instance the degree of intra and intersexual competition for resources, may do a better job of explaining the presence of female song in certain temperate zone species. A more exhaustive examination of the natural history characteristics related to the putative selective forces shaping female song may shed more light on the presence and absence of this classically male trait in female organisms.

CHAPTER 1 ACKNOWLEDGEMENTS

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APPENDICES

APPENDIX A

Supplemental figures for Chapter 1

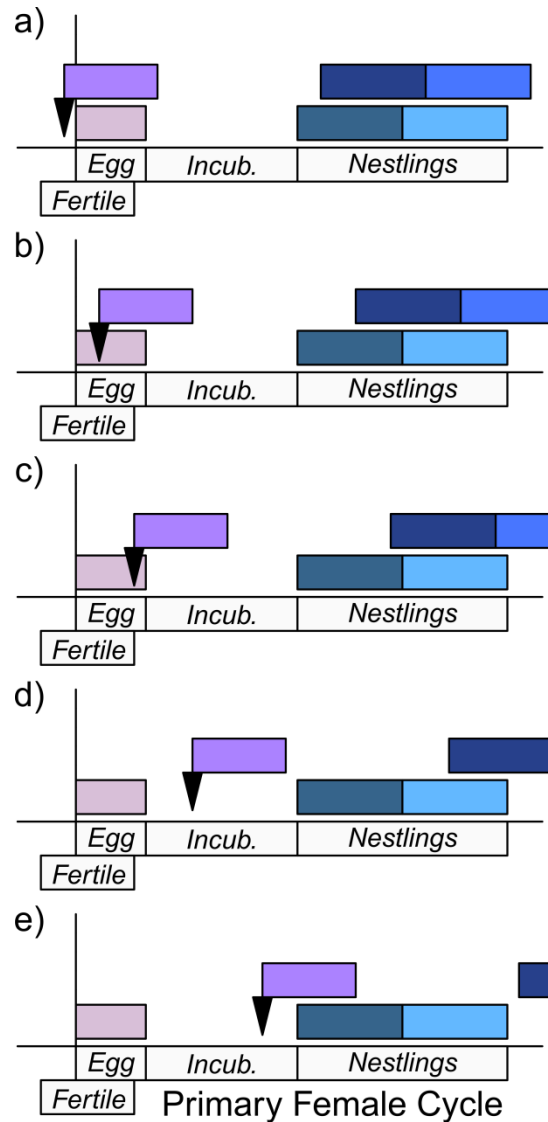


Figure 1.6: Periods of conflict between primary and secondary female house wrens settling on days -1 (a), 2 (b), 5 (c), 10 (d) and 16 (e) in the primary female's nesting cycle. The arrow represents the day the secondary female joins the territory. Bottom purple bars represent the period when the primary female's eggs are vulnerable to ovicide. Upper purple bars represent the period when the secondary female will engage in ovicide. Secondary females are the largest threat when these purple bars overlap. Bottom blue bars represent the period between hatching and fledging for the primary female's nestlings. The darker half of the bar represents the period of most rapid nestling growth during which male provisioning is needed most. Upper blue bars represent the nestling period for secondary females. Secondary females are the largest threat to

Figure 1.6 (cont'd): parental care when the dark halves of these bars overlap. Here we assume that secondary females need several days to finish lining the nest and develop their eggs after settling on a territory.

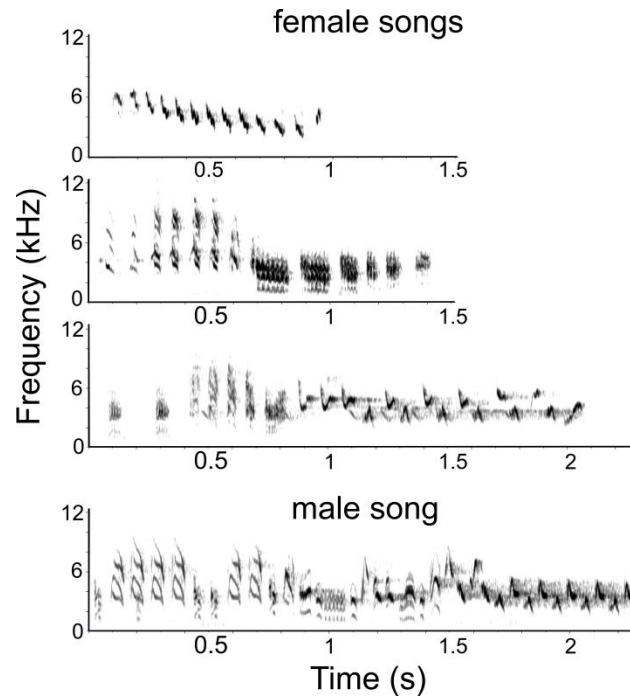


Figure 1.7: Three examples of female house wren song (top) compared to a male house wren song (bottom). Spectrograms were prepared using Raven Pro v.1.4 (Bioacoustics Research Program, 2011). Sample recordings are available at <http://www.xeno-canto.org> (XC263716, XC263718, XC263719, XC263720).

APPENDIX B

Supplemental tables for Chapter 1

Table 1.2: Timing of spontaneous female songs not included in this analysis

Day	Context	No. of visits with songs ^a
0	Control playback/post-playback ^a	5
2	Control playback/post-playback	3
3	Control playback/post-playback	4
4	Control playback/post-playback	3
20	Feeding observation ^b	1
23	Feeding observation	2
24	Feeding observation	4
25	Feeding observation	1
26	Feeding observation	1
26	Returning nestlings to the nest	1

^a Including these observations does not change the significance of the results our analysis. Observations were excluded to standardize observation time and disturbance level.

^b Song was observed during the heterospecific playback or during the silent post-playback period of control trials, outside of the initial 5 min included in the analysis.

^c During feeding observations, a human observer silently watched the nest for 30 min from 20 m away without ever approaching the nestbox. These observations were excluded from the analysis because they represent a low level of disturbance relative to the moderate level of disturbance in the contexts included in the analysis. In all but two of these feeding observations with song, song occurred after 5 min.

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CHAPTER TWO

Female house wrens use a low-amplitude call as an aggressive signal

Cara A. Krieg & Alexandra D. Burnett

ABSTRACT

The majority of work on aggressive signaling has focused on male-male contests. Although females in many species compete over important resources, female signals are understudied. In house wrens (*Troglodytes aedon*), females compete with other females to obtain and protect nesting cavities and secure paternal care. We show that a low-amplitude call, the HI call, is an aggressive signal used by female house wrens. Using playback of simulated female intruders, we found that females used HI calls significantly more often during female playback compared to the silent pre-playback period. Additionally, we found that HI calls predicted future aggressive behavior and were given significantly more often in the minute preceding physical attack. In comparison, song rates did not predict female aggression score or future attacks after accounting for the effect of HI calls. Finally, we found that listening females responded significantly more aggressively to female playback containing HI calls, suggesting the reliability of HI calls as an aggressive signal may be maintained by a receiver retaliation rule. HI calls bear a striking resemblance to the low-amplitude songs of many male songbirds, both in terms of acoustic structure and social context. The nature of female house wren competition, however, may allow females to avoid some of the trade-offs faced by males using low-amplitude signals. To our knowledge, this is the first low-amplitude aggressive signal reported in a female songbird.

INTRODUCTION

Fights to the death are quite rare in nature (Enquist & Leimar 1990). Most animal contests involve an exchange of signals before they come to blows. Resolving conflicts through signals can benefit both senders and receivers (Enquist 1985, Hurd & Enquist 2001). However, when contests are decided without a physical fight, selection will favor weaker individuals that bluff and send intimidating signals (Maynard Smith 1974, Maynard Smith & Parker 1976, Dawkins & Krebs 1978). Researchers studying signals face the challenge of showing how the honesty of those signals is maintained over evolutionary time.

Information relevant to aggressive contests comes in two forms: information about fighting ability and information about aggressive intent (Enquist 1985, Searcy & Beecher 2009, Hurd & Enquist 2005). Signals of fighting ability are typically inflexible signals for which honesty is maintained by physical constraints, such as the size of visible weapons or low frequency vocalizations as proxies for body size (Clutton-Brock & Albon 1978, Davies & Halliday 1978). Flexible signals of aggressive motivation provide many more opportunities for senders to cheat and have thus attracted much attention from both theoreticians and empiricists alike (Enquist 1985, Poole 1989, Waas 1991, Vehrencamp 2000, Hurd & Enquist 2001, Számadó 2003, Laidre & Vehrencamp 2008).

Searcy and Beecher (2009) described three criteria that must be satisfied to establish a given signal is one of aggressive intent. First, aggressive signals should be given more often in aggressive contexts (context criterion). Second, signals of intent should also predict future aggressive escalation by the sender (predictive criterion), since both signals of escalation and submission should increase during agonism. Third, effective aggressive signals should alter the behavior of intended receivers at least some of the time (response criterion). In practice, the

interpretation of these receiver responses can be difficult. Receivers may respond *more* intensely to aggressive signals because they indicate a large threat that must be deterred forcefully (Nelson & Croner 1991). However, receivers may respond *less* intensely to aggressive signals to avoid a costly conflict with a much stronger rival (Järvi et al. 1980). A signal can only be definitively considered an aggressive signal if it meets the context, predictive, and response criteria (Searcy & Beecher 2009). Most putative aggressive signals studied to date only satisfy one or two of these criteria (Searcy & Beecher 2009).

The majority of studies on aggressive signaling have focused on males rather than females. Male reproductive success is often largely determined by access to a limited number of fertile females or the resources used to attract them (Shuster & Wade 2003). It is unsurprising, then, that most signaling studies focus on male-male contests over these critical resources. Male birds, in particular, are a favorite model for those studying aggressive signals of intent. Low-amplitude songs (song sparrows: Searcy et al. 2006, Templeton et al. 2012, Anderson et al. 2012; swamp sparrows: Ballentine et al. 2008), low-amplitude calls (corncrakes: Ręk & Osiejuk 2011), gargle calls (black-capped chickadees: Baker et al. 1991, Baker et al. 2012), song type matching (Western populations of song sparrows: Akçay et al. 2013, Burt et al. 2001, Vehrencamp 2001), and wing waves (swamp sparrows: Ballentine et al. 2008, Anderson et al. 2013) have all been shown to be aggressive signals in birds that fulfill Searcy and Beecher's three criteria. Low-amplitude vocalizations are particularly intriguing since males in multiple unrelated species appear to have converged on a similar signal form.

In many animals, however, both males *and* females compete for important resources. In house wrens (*Troglodytes aedon*), both males and females compete for access to suitable nesting sites (Johnson & Kermott 1990, Johnson & Searcy 1996). House wrens are small (10-12g),

migratory songbirds that nest in secondary cavities. Even after a breeding pair has secured a territory, conspecifics threaten reproduction through interference and usurpation attempts. Both male and female house wrens destroy any eggs they can find nearby prior to their own clutch initiation (Belles-Isles & Picman 1986). Ovicide also occurs in conjunction with attempted territory takeovers. Between 4-15% of the eggs at our study site are lost to wren interference each year (CA Krieg, unpublished data). Intruding females represent an additional threat to resident females since they may settle as a secondary female (Kendeigh 1941). Therefore, intruding females may threaten resident female interests by usurping her nesting cavity and place in the breeding partnership, destroying some or all of her eggs, and/or diverting a portion of male provisioning effort once her nestlings hatch. We investigated whether the low-amplitude HI call described below is an aggressive signal of intent used by female house wrens in conflicts with female intruders.

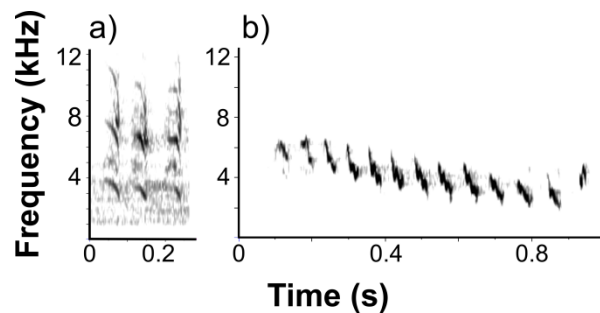


Figure 2.1: (a) A spectrogram of three HI calls and (b) a song produced by the same female house wren. Darkness is not indicative of absolute differences in amplitude between (a) and (b).

The HI call is a low-amplitude, high frequency call note produced by house wrens (“series of squeaky notes”: Kendeigh 1941; “high-frequency squeaks”: Johnson & Kermott 1991). Like soft song in males of several other species (Reichard & Welklin 2015), HI is associated with aggressive as well as courtship contexts. We often observe HI in courtship interactions that result in copulation. However, we also frequently hear HI calls in response to

conspecific playback. HI calls are quieter than both female song and male broadcast song. We have not measured amplitude directly from birds in the field. However, we do have a handful of songs and HI calls recorded from females vocalizing at very close distances to the human observer (1-2 m). When we played back clean, unmanipulated recordings of female songs that were given immediately before a series of HI calls, we found that female songs ranged from 70-75 dB and HI calls ranged from 51-54 dB as measured by a digital sound level meter from 1 m away. Although this measure does not accurately assess absolute amplitude, it does suggest that HI calls are produced at notably softer amplitudes than song. A single HI contains several harmonic overtones and is characterized by a relatively short duration (average 0.05 seconds) as well as some of the highest frequencies and widest frequency spreads of any female house wren vocalization we have recorded thus far (~3-11 kHz; Figure 2.1). Both male and female house wrens appear to produce HI calls, although there may be subtle sex-specific variations in structure that we have not captured. We have previously reported that female house wrens use song when engaging with intruding conspecific (Krieg & Getty 2016). Our previous work also suggested that playback of HI calls may provoke responses from listening females.

Using recordings of focal females made during playback of simulated female intruders, we investigate whether HI is an aggressive signal in female house wrens that fulfills Searcy and Beecher's (2009) criteria. First, we asked whether HI calls were preferentially used in aggressive contexts (context criterion). Second, we asked whether the use of HI calls predicted future aggressive escalation (predictive criterion). Third, we asked whether HI calls produced measurable responses in receivers (response criterion). Finally, we use results from this playback experiment and information on the transmission of HI calls through the environment to discuss potential mechanisms that may maintain the reliability of HI as an aggressive signal.

METHODS

Study Site and Study System

We conducted this work on the house wren population breeding at the Lux Arbor Reserve of the Kellogg Biological Station in Barry County, MI (42° 29' N, 85° 28' W). Lux Arbor is a 529 hectare protected plot of land containing wetlands, mixed forests, grasslands, and small agricultural fields. For the last 15 years, we have set up nestboxes on 59 territories that house wrens occupy every year from early May to mid August (Dubois & Getty 2003). Fifty-six females in 2012, 69 females in 2013, and 31 females in 2014 nested at the study site. Each female was captured at least once and given a United States Fish and Wildlife band and three color bands to allow for individual identification. We also measured female mass to the nearest 0.1 g and tarsus length to the nearest 0.01 mm. We calculated female condition as the residual after regressing tarsus onto mass for all females with nesting stage included as a covariate to account for systematic differences in female mass between incubation and pre-incubation (for similar approach see DeMory et al. 2010). Some females were captured in mist nets prior to incubation either after playback trials or at least 3 days before a trial. The remaining females were trapped on the nest during incubation.

Playback Stimuli

To assay female aggressiveness, we presented females with playback of female house wren songs and a separate playback of heterospecific songs. Each female heard one stimulus for the set being used that year (discussed below). Each playback stimulus consisted of recordings of 6 high signal-to-noise-ratio songs given by a single known individual separated by 5 seconds of silence to approximate pauses during natural singing bouts. Heterospecific stimuli contained songs of various cup-nesting birds (*Turdus migratorius*, *Dumetella carolinensis*, *Pipilo*

erythrophthalmus, *Melospiza melodia*, *Spizella passerina*, and *Spizella pusilla*). All background noise below 1 kHz was filtered out and each song was standardized to a peak amplitude of 1.4 kU using Raven Pro v1.4 (Bioacoustics Research Program 2011) (“U” is a dimensionless sample unit in Raven Pro). Each stimulus was repeated for 5 minutes. All tracks were played back at a peak amplitude of 70-75 dB measured at 1 m using an Extech digital sound level meter (model 407732).

Table 2.1: The female playback stimuli used in each year.

2012	2013	2014
f-a6 ^a		
f-17 ^b	f-17b1	f-17b1
	f-17b2 ^a	
f-a2 ^a		
f-21	f-21	f-21
f-c4-HI ^c	f-c4	f-c4-HI ^c
f-23-HI ^c	f-23 ^d	
	f-c1	f-c1
	f-42	f-42
	f-18	f-18
	f-38	f-38
	f-a1	f-a1
	f-27	f-27
	f-17(13) ^a	
	f-14 ^a	
	f-25 ^a	
	f-c5 ^a	

^a Stimulus was discontinued because it produced low responses during trials

^b Recordings from this same bird were used to create two stimuli in 2013 (f-17b1, f-17b2)

^c Stimulus contains HI calls

^d Stimulus was discontinued because it was used as a mist netting lure in 2014.

We used a different set of stimuli each year, depending on the number of recordings available and their history of provoking responses (Table 2.1). These playback trials were part of a separate experiment aimed at assessing female aggressiveness (Krieg, unpublished data). Therefore, if a stimulus provoked weak responses, we did not use that stimulus again in the next

year. Within each year, however, we randomly assigned each bird a stimulus from that year's set. Dropping the discontinued stimuli from the response criterion analysis (see "*Analysis: Response Criterion*" below) did not change conclusions regarding HI calls. Females were never played their own song, the songs of a neighbor sharing a territory boundary, or a stimulus they had heard in a previous year. Ideally each female would have been tested with a unique stimulus, however, we were limited by the number of high quality recordings available. In 2012, two of the stimuli contained several HI calls that the stimulus bird had given before or after their songs. At the time, we were unsure whether these calls were part of the song itself, so we included them in the stimuli. In 2013, we filtered out the HI calls. In each year, females also heard one of six possible heterospecific stimuli.

Playback experiment

For each trial, we placed a speaker attached to a tripod within 2m of the nestbox. For playback female trials, a wood block carved and painted to roughly resemble a house wren was placed on top of the tripod to provide a potential target for aggression (2012-2013). We were concerned that having a model present during the pre-playback period of the female but not heterospecific trials might alter responses. Therefore, in 2014 the bird model was replaced with a wooden egg of the same size and color that was presented during heterospecific and female trials. Aggressive responses, HI calls, and songs to heterospecific and female trials did not vary significantly based on model type (Mann-Whitney tests; aggression score: heterospecific: $W = 1006$, $P = 0.71$; female: $W = 1148$, $P = 0.82$; HI calls: heterospecific: $W = 1078$, $P = 0.08$; female: $W = 1334.5$, $P = 0.28$; songs: heterospecific: $W = 1056$, $P = 0.12$; female: $W = 1327$, $P = 0.28$).

Each trial started with a 5 minute period of silence, continued to a 5 minute period of playback, and concluded with a final 5 minute period of silence. An observer seated 20 meters away recorded all vocalizations using a Marantz PMD 660 (2012-2013)/661 (2014) solid state digital recorder attached to a Sennheiser ME62 omnidirectional microphone and a Mineroff parabolic reflector. This observer narrated all physical attacks and location changes by the focal female aided by flags hung at 1m and 5m. The sex of responding birds was easy to determine using color bands and sex-specific vocalizations. All birds were tested between sunrise and 12:30 EST within the first four days of the female's egg laying period. Heterospecific and female treatment trials were conducted on separated days with trial order assigned randomly. As part of another experiment, females also heard a male trial during this time (Krieg & Getty 2016). Exposure to the male house wren playback trial did not affect the aggression score, female songs during playback, or HI calls during playback in the female trial (Mann-Whitney U tests; aggression: $W = 1233$, $P = 0.08$; songs: $W = 1393$, $P = 0.34$; HI calls: $W = 1404$, $P = 0.40$). We tested 51 females in 2012, 55 females in 2013, and 28 females in 2014. We eliminated any trials where the female was never seen or heard during the entire 15 minutes (13 female trials, 23 heterospecific trials), leaving 117 female trials and 109 heterospecific trials on 114 different females in the final data set. For additional details on this playback experiment see Krieg and Getty (2016).

Aggression Score

Many studies on avian aggression use either physical aggression as a direct measure of aggressive response or a principle component analysis that combines several proxies for aggressiveness into a single variable (e.g., Järvisjö et al. 2013, Akçay et al. 2013). We chose instead to calculate an aggression score based directly on four behaviors that are commonly

measured in playback studies: attacks, dives, time spent within 1m of the tripod and speaker, and latency to approach the speaker within 5m. This approach has several advantages. First, a small number of females either attacked or dove at the set-up (22.9% female trials), but non-attacking birds still varied in how strongly they responded. The aggression score allows us to examine variation in non-attackers. Second, the interpretation of the aggression score is more straightforward than the results from a principle component analysis. It was impossible for a bird to obtain a score greater than 2 without either diving or attacking. Third, the aggression score approach allows us to weight the importance of each behavior in our assessment of aggressiveness. This is likely more realistic since physically attacking the set-up is clearly more aggressive than simply advancing to a close distance. Fourth, the aggression score could reflect the non-aggressive responses of some birds that avoided the set-up when playback was present. We calculated aggression score using the formula below:

$$\text{Aggression score} = 3 * (\# \text{ of attacks}) + 2 * (\# \text{ of dives}) + 1 * ((300 - \text{seconds to approach within 5m}) / 300) + 1 * ((\text{seconds within 1m during playback} - \text{seconds within 1m during pre-play}) / 300)$$

We define an attack as making actual physical contact with either the speaker or the model and a dive as swooping rapidly within several centimeters of the set-up. We chose to subtract the time spent within 1m during the pre-playback period from the time spent within 1m during the playback period to both control for birds that spend more time near the nestbox in non-aggressive situations as well as capture those that retreated during active playback. Results using

aggression scores are qualitatively similar to results using the number of dives and attacks in all analyses below.

Transmission Distance Experiment

To determine how the HI call degrades over distance in comparison to female songs, we recorded a loop of female songs and HI calls as we walked along two 40 m transects away from playback. While we did not measure amplitude of HI calls directly from singing birds in this experiment, this approach allows us to roughly determine how HI calls propagate through background noise compared to female songs as determined by the acoustic properties of each vocalization.

We constructed this playback using five female songs and five HI calling bouts. Each HI calling bout was originally recorded at the same time, from the same distance, and using the same volume settings as a corresponding female song in the playback loop. From this loop we haphazardly selected 5 female song syllables, 5 HI calls, and 5 silent gaps for further analysis. At the beginning of this loop we placed a loud cardinal song which allowed us to determine the start of each repetition even at a distance.

We conducted this test over two perpendicular transects. Using the same speaker and recorder as playback trials, we recorded at 5 m, 10 m, 20 m, 30 m and 40 m from the speaker in one continuous track without changing volume settings. We allowed the loop to complete for 5 repetitions before moving to the next distance. These recordings were made in late August at approximately 10:00 EST on a house wren territory with typical forest vegetation on a day with typical weather conditions.

For analysis, we then selected the 3 repetitions for each distance and transect with the least background noise and used Raven Pro v1.4 to measure the root mean squared amplitude for

each selection. For song syllables and HI calls, we measured amplitude only over the frequencies at which the selection was visible on the spectrogram in the original recording. For the silent periods, we measured amplitude over all frequencies. For each selection, the measurements over the 3 repetitions were averaged together to get a single measure at each distance. When viewing these results, readers should note that house wren perceptual capabilities might differ from the ability of the recorder to detect these sounds.

Analysis

Context Criterion. To determine whether HI calls are given more often in aggressive contexts, we compared the number of HI calls given during the pre-playback period and the playback period of the female trials. To avoid the possibility that females give HI calls in response to recordings with HI calls, we eliminated all trials using stimuli with HI calls for this analysis (final $N = 98$ trials). We also included a comparison of the pre-playback and playback period of the heterospecific trials to show this increase is not merely an artifact of the playback set-up ($N = 109$). We used Wilcoxon signed-rank tests with Holm's familywise error rate corrections to the P values to correct for multiple comparisons (Holm 1979). We conducted this and all subsequent analyses using R version 3.0.2 (R Core Team 2013; package glmmADMB: Fournier et al. 2012; Skaug et al. 2015; package MuMIn: Barton 2015; package MASS: Venables & Ripley 2002; package car: Fox & Weisberg 2011).

Predictive Criterion. To determine whether HI calls predict escalation, we examined two questions: 1. Do HI calls given during playback predict the aggression score of the female giving the HI call during that trial?, 2. Do HI calls predict future physical attacks by the signaling female? To analyze the aggression score data, we used a generalized linear model with a gamma distribution that included HI calls and songs as covariates ($N = 117$ female trials). We

log transformed all variables to satisfy test assumptions. VIF scores were below 1.6, indicating no issues with multicollinearity. Since generalized linear models do not produce R^2 values used in traditional linear models, we calculated pseudo- R^2 values based on a likelihood ratio test (Magee 1990).

To examine the predictive ability of HI calls, we followed the procedure of Searcy et al. 2006. We randomly paired each trial with an attack with 5-6 randomly selected non-attack trials (no re-sampling) with the constraint that the non-attacking birds must have responded to the playback by the time the attack occurred in the paired attack trial. This avoids comparing measuring periods when the focal female was absent which would bias the non-attack trials toward low levels of vocal response. In 8 non-attack trials, females did not respond until after all the attack windows. We did not include these trials. For this analysis, we considered both dives and attacks as a form of physical attack. We then used a binomial generalized linear model with HI calls and songs in the minute before attack included as covariates to determine whether these vocal behaviors predicted subsequent attack. To test how well a model with only HI calls in the minute before the attack period correctly classified birds into attackers and non-attackers, we used a jackknifed procedure with a quadratic discriminant function analysis (Searcy et al. 2006). If birds attacked more than once during the trial, we only included the first attack. Overall, this analysis examined 16 attack trials and 93 corresponding non-attack trials.

Response Criterion. To determine whether HI calls are salient to receivers, we examined which playback characteristics are most important in predicting the aggressive response of listening birds. First, we used a principle component analysis to summarize several acoustic parameters of the playback stimuli, since many of these measures were highly correlated. For each female playback stimulus, we measured 7 variables using cursors on

spectrograms produced in Raven. (1) *Average syllable type switches*: Each syllable in female songs falls into one of 3 categories. Tonal syllables are pure tones that form a continuous trace on the spectrogram. Multi-tonal syllables have two or more clearly visible harmonics. Buzz syllables span a wider range of frequencies than tones, lack clear overtones, and have a harsh quality (see Appendix). For each song in the stimulus, we counted the number of switches between these syllable types and then averaged them together for the whole stimulus. We also measured (2) the *average song duration*, (3) *average syllables per second*, (4) *average highest frequency of each song*, (5) *average frequency spread of each song*, (6) *average standard deviation of frequency spread of each syllable in each song* as an approximation of variation in song shape, and (7) *average syllable type diversity of each song* using Shannon's diversity index (see Appendix). We also took the time in seconds containing HI calls and divided it by the total time containing any vocalization in each stimulus (*proportion of stimulus that was HI calls*). We did not include this variable in the final principle component analysis since it was our primary variable of interest and did not map cleanly onto any single principle component. Because of the way we constructed playbacks, stimuli with longer songs also contained more total time of vocalizations.

Then we used a backward stepwise model selection based on Akaike information criterion to determine which playback characteristics entered a linear model explaining the average aggressive score response of receivers (candidate variables: PC1, PC2, PC3, PC4, the proportion of the playback vocalization time that was spent on HI calls). Pseudo-replication is a concern when responses to a single exemplar from a given class are used to make inferences about responses to all representatives from that class. Therefore, we followed the advice of

Kroodsma et al. (2001) and averaged the responses of all females who heard each stimulus before running these models. Therefore, the sample size for this analysis was 21.

Females should only use aggressive signals if they sometimes cause receivers to retreat before a physical conflict. Theory predicts that, following signals of aggressive intent, some receivers should remain in the conflict (strong and/or motivated individuals) whereas some receivers should retreat (weak and/or unmotivated individuals), even though this prediction is rarely assessed in empirical studies (Enquist 1985, Hurd 1997). We asked whether HI calls did in fact cause some receivers to temporarily retreat. We selected 6 HI calls separated by at least 20 seconds within the two playback stimuli that contained them (Table 2.1). We calculated each female's average distance from the playback speaker in the 10 seconds before and after the playback of the HI call (16 trials). We then calculated average distances for the closest corresponding song using the same playback recordings with the HI calls removed (6 trials). We only calculated these distances if the female's location was known for the entire 20 seconds. Using three Mann-Whitney U tests with Holm's *P*-value adjustments, we compared whether the proportion of times females retreated, stayed at the same distance, or approached the speaker differed for birds that had just heard a HI call compared to birds that had just heard a song.

Ethical Note

This research was approved by the Michigan State University's Institutional Animal Care and Use (IACUC) panel. Field work was conducted under appropriate federal and state permits (MSU IACUC Animal Use Form numbers 04/11/076-00, 03/14-062-00; U. S. Fish and Wildlife Service Federal Banding Permit number 23587; Michigan Department of Natural Resources Scientific Collectors Permit number SC 1432, U. S. Fish and Wildlife Service Permit number

MB01550B-1). No females abandoned territories following our tests. No birds suffered adverse effects during capture and measurement.

RESULTS

Context Criterion

Female house wrens used HI calls significantly more often in aggressive contexts (Figure 2.2). Females gave significantly more HI calls once playback of a simulated female intruder began (Wilcoxon signed-rank test: $N = 98$, $V = 955.5$, $P < 0.001$). Females did not increase their HI call rate between the pre-playback and playback period of the heterospecific trials, suggesting that the playback set-up alone did not provoke this response (Wilcoxon signed-rank test: $N = 109$, $V = 91$, $P = 0.61$). Furthermore, of the HI calls given during female trials, 87.8% were given within 5 m of the speaker set-up. If the simulation had been a real intruder, then it is likely the HI call would have been perceived at these close distances.

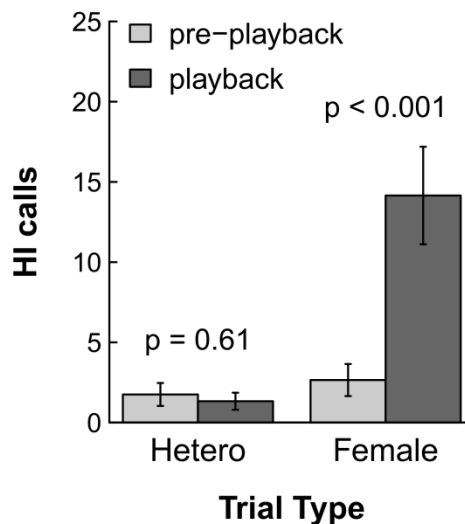


Figure 2.2: The mean number of HI calls ($\pm$ SE) during the pre-playback and playback period of heterospecific ($N = 109$) and female ($N = 98$) trials. Trials that used playback stimuli with HI calls were omitted. P -values are results from Wilcoxon signed-rank tests with Holm's P -value corrections.

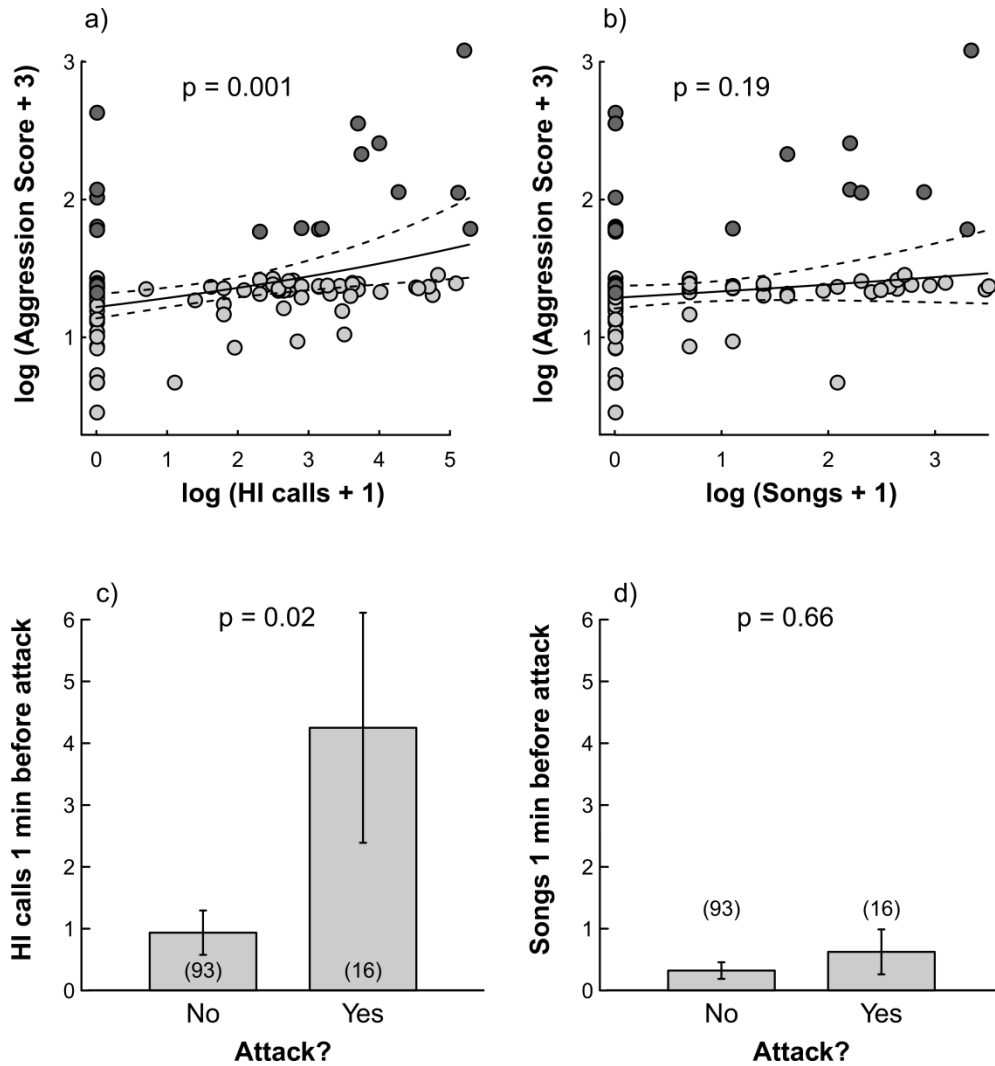


Figure 2.3: (a), (b) The relationship between the number of HI calls and songs given during female playback and the aggression score the listening female received. Lines represent predicted values ($\pm$ 95% CI) from a gamma generalized linear model containing HI calls and songs as covariates ($N = 117$). Both variables were shifted to include only positive numbers and then log transformed to meet model assumptions. Darkened points indicate trials where females dove at or attacked the set-up. (c), (d) The number of HI calls and songs ($\pm$ SE) given 1 minute prior to attack compared to the same time period for randomly chosen non-attackers. P -values are from a binomial generalized linear model containing HI calls and songs as covariates. Numbers in parentheses indicate the number of trials in each category.

Predictive Criterion

HI calls predicted future aggressive behavior by the calling female (Figure 2.3). Females that gave more HI calls during female playback achieved significantly higher aggression scores

during that trial after controlling for the effect of female song rate, indicating that they approached faster, spent more time in very close proximity to the set-up, and physically engaged with the simulated intruder more often (Figure 2.3a; gamma generalized linear model: $N = 117$, estimate $\pm$ SE = -0.04 ± 0.01 , $\chi^2 = 10.2$, $P = 0.001$, likelihood ratio pseudo- $R^2 = 0.3$). In contrast, female song rate did not predict female aggression scores after controlling for the significant effect of HI calls (Figure 2.3b; estimate $\pm$ SE = -0.02 ± 0.02 , $\chi^2 = 1.7$, $P = 0.19$). Furthermore, females that dove at or attacked the set-up give significantly more HI calls in the minute preceding attack than non-attacking females over the same time period (Figure 2.3c; binomial generalized linear model: $N = 109$, estimate $\pm$ SE = 0.13 ± 0.06 , $\chi^2 = 5.23$, $P = 0.022$, model likelihood ratio pseudo- $R^2 = 0.09$). Song rate did not predict attack after controlling for the effect of HI calls (Figure 2.3d; estimate $\pm$ SE = -0.11 ± 0.25 , $\chi^2 = 0.20$, $P = 0.66$). A quadratic discriminant function analysis using a jackknife procedure correctly classified 93.5% of non-attacking females and 31.3% of attacking females (overall 84.4% correctly classified).

Although HI calls generally predicted escalation, the reliability of this signal was not perfect. In 10 trials, females gave HI calls but did not attack (10.8% non-attacks). In 11 trials, females attacked without giving any HI calls in the minute preceding attack (68.8% of attacks). Only one of these females sang in the minute prior to attack. However, in 4 of these trials, females attacked as their first response to playback and another 2 attacked in less than 20 seconds from their first response. This may represent a different category of response. Overall, 31.3% of attacking females gave HI calls prior to attack, 37.5% escalated immediately to attack, and 31.3% had ample opportunity to signal but did not do so.

Table 2.2: Principle component loadings of acoustic variables from playback stimuli.

Variable	PC1	PC2	PC3	PC4
switches between syllable types	0.41	-0.38	0.003	-0.46
song duration	0.31	-0.26	0.76	0.44
syllables per second	0.05	-0.65	-0.55	0.37
highest frequency	0.40	0.37	-0.16	0.41
frequency spread	0.34	0.48	-0.22	0.04
std frequency spread (shape)	0.47	-0.04	-0.20	0.19
syllable type diversity	0.48	-0.04	0.05	-0.50
Cumulative variance explained	0.55	0.78	0.88	0.94

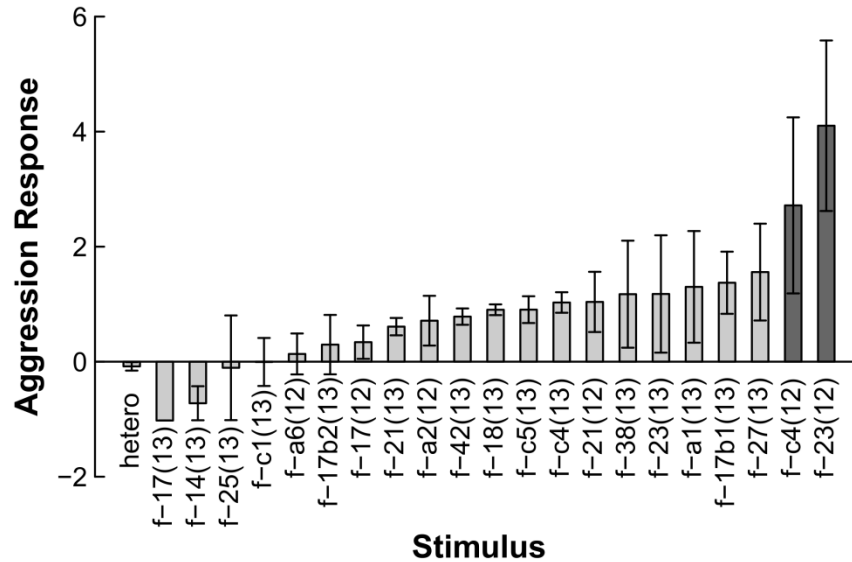


Figure 2.4: The average aggression score response of female house wrens that heard each female song stimulus ($\pm$ SE) with the response to heterospecific trials included for comparison. Darkened bars indicate stimuli that included HI calls. The number in parentheses indicates whether the stimulus was initially created for the 2012 or 2013 stimuli set.

Response Criterion

Females responded differently to HI calls than to other female vocalizations. The aggressive response of listening females varied depending on the stimulus they heard (Figure 2.4; gamma generalized linear model, $N = 117$, $\chi^2 = 33.42$, $P = 0.03$, likelihood-ratio pseudo $R^2 = 0.41$). The two stimuli that provoked the strongest average response both contained HI calls

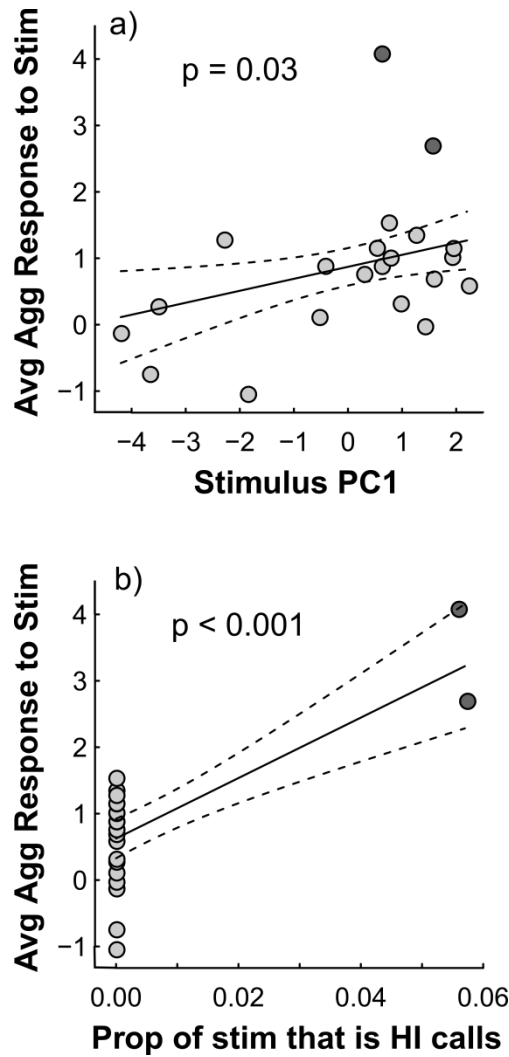


Figure 2.5: The relationship between the PC1 of the playback stimulus (a), the proportion of stimulus vocalization time in seconds that was HI calls (b) and the average aggressive response of listening birds. Dark points indicate stimuli that contain HI calls. Lines represent predicted values from a linear model $\pm$ 95% CIs with PC1 and proportion of stimulus that was HI calls as covariates ($N = 21$). Points indicate the averaged response of all trials using that playback stimulus.

(Figure 2.4). Only principle component 1 and the proportion of stimulus vocalization time that contained HI calls entered the final model. Females responded significantly more aggressively to stimuli with higher values of PC1 after controlling for the effect of HI calls (Figure 2.5a; linear model; $N = 21$, estimate $\pm$ SE = 0.18 ± 0.08 , $F = 5.75$, $P = 0.028$; model $R^2 = 0.65$).

Recordings with high values of PC1 contain songs that are overall acoustically more variable

(Table 2.2). They have a higher diversity of syllable types, frequent switches between different syllable types, and a higher standard deviation in frequency spread, indicating more variation in song shape. However, the proportion of stimulus vocalization time that contained HI calls was the overall strongest predictor of female aggression (Figure 2.5b; estimate $\pm$ SE = 45.51 ± 8.65 , $F = 11.61$, $P < 0.0001$). Figure 2.5a clearly shows that the two stimuli containing HI calls provoked a much higher aggressive response than stimuli with similar PC1 values.

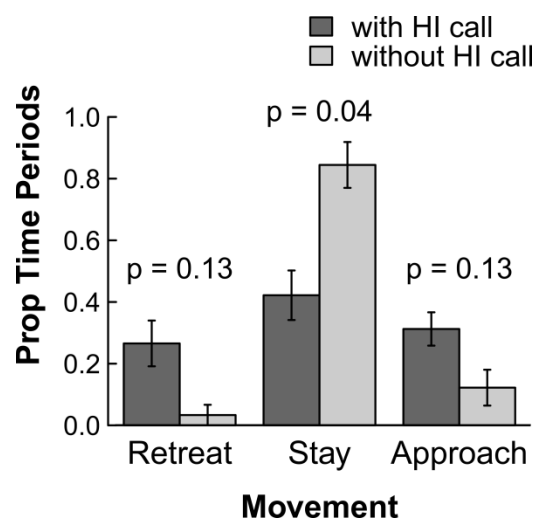


Figure 2.6: The proportion of times ($\pm$ SE) females retreated from, stayed at the same distance to, or approached the playback speaker in the 10 seconds following playback of a HI call ($N = 16$) compared to playback of the closest corresponding song from playback of the same stimulus female with the HI calls removed ($N = 6$). P -values are from Mann-Whitney U tests with Holm's P -value adjustments to correct for multiple comparisons.

On average, stimuli containing HI calls provoke a stronger aggressive response from listening females. However, as predicted by theory, HI calls may cause a subset of birds to retreat from the signaling female. Females were significantly less likely to remain at the same distance to the speaker in the 10 seconds immediately following playback of a HI call than they were immediately following female songs in similar playback tracks (Figure 2.6, Mann-Whitney U test; $N = 16$ HI trials, 6 no HI trials; $W = 14$, $P = 0.038$). Although the differences were not

significant, females tended to approach more often and retreat more often following HI calls compared to song (Mann-Whitney U tests; approach: $W = 72.5$, $P = 0.127$; retreat: $W = 72$, $P = 0.127$).

Additional Information on HI Calls

HI calls are not used preferentially by birds of a given size or physical condition. Neither condition nor mass significantly affected the number of HI calls given during female playback (negative binomial generalized linear models; condition: $z = 0.4$, $P = 0.67$; mass: $z = 0.39$, $P = 0.69$). Furthermore, 82% of females were recorded producing HI calls at some point in this study, suggesting most if not all birds are capable of this behavior.

Although we sometimes hear HI calls in the context of courtship, the HI calls given by females during female trials do not appear to be exclusively directed towards mates. For females that gave HI calls at some point during the female trial, 48% gave at least some HI calls when their mate was out of sight. Furthermore, the number of HI calls females gave during female playback was not correlated with the amount of time the male was present (Pearson's $r = 0.03$, $P = 0.73$). We cannot rule out the possibility that pair communication happens during our trials. However, we believe the fact these calls predict subsequent attack suggests they are sometimes directed at female rivals as well. Male house wrens may also use HI calls during aggressive interactions, but testing this hypothesis was outside the scope of this study.

As a signal, HI is limited in its ability to transmit to distant receivers, and thus similar to other low-amplitude signals. Compared to female song, the discriminability of HI calls over background noise degrades quickly as the distance from the sender increases (Figure 2.7). Therefore, the intended receivers of HI calls are likely located in relatively close proximity to senders.

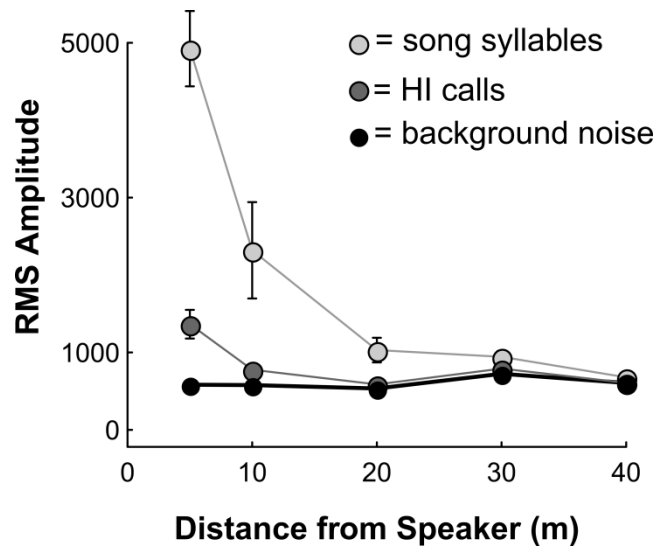


Figure 2.7: The degradation of the root mean squared amplitude of HI calls and female song syllables compared to background noise as distance from the speaker increases. Each point represents the average of the same five selections ($\pm$ SE) for each of two transects. RMS amplitude is measured in dimensionless units.

DISCUSSION

Our results show that a low-amplitude call, the HI call, is an aggressive signal of intent in female house wrens. HI calls satisfied all three criteria outlined by Searcy and Beecher (2009) to establish a signal as aggressive in nature. Females gave significantly more HI calls during playback of female conspecifics compared to the silent pre-playback period (context criterion). HI calls predicted future escalation with females that attacked giving significantly more HIs in the minute prior to attack than females that did not attack (predictive criterion). HI calls were more informative than female song rate in predicting female aggressions scores as well as whether females attacked. Finally, receivers, on average, responded significantly more aggressively to female playback containing HI calls (response criterion). HI calls may cause a subset of females to temporarily retreat from playback, although full de-escalation was likely

inhibited in this experiment due to the non-interactive nature of the playback (Laidre & Vehrencamp 2008) and high-stakes nature of a challenge near the nest.

Species with frequent conflict between males evolve signaling systems to mediate conflicts (Briffa & Sneddon 2010). Although females compete with other females for important breeding resources in many species, female competition has received considerably less research attention (Clutton-Brock 2009, Clutton-Brock & Huchard 2013). Here we show a parallel signaling system in female house wrens that compete for limited nesting cavities and exclusive access to male parental care. To our knowledge, this is the first documentation of a low-amplitude aggressive signal in females, a common signaling form in males.

Signal Reliability

Our data suggest the reliability of HI calls as an aggressive signal might be stabilized by a receiver retaliation rule. Reliability of aggressive signals can be maintained by various mechanisms depending on signal type. The honesty of index signals and quality handicaps (also known as “*cost-added signals*”) are both maintained by physical limits imposed either by signal form (e.g. low frequency sounds correlated with body size), by the costs associated with bearing the signal (e. g. long ornamented tails increasing predation risk) (Vehrencamp 2000, Maynard Smith & Harper 1995) or by differences in the benefits (Getty 2006). HI calls do not appear to fit these criteria. The majority of females were capable of producing HI calls, and HI production had no relationship to the mass or condition of the sender. We believe instead that a receiver retaliation rule most likely stabilizes the reliability of HI as a signal, given our data. In these systems, receivers that assess themselves as stronger or as strong as the signaling bird frequently attack following the aggressive signal. Weaker birds that bluff and send the aggressive signal will thus frequently engage in costly fights with individuals stronger than themselves. Therefore,

the honesty of the aggressive signal is not maintained by the intrinsic properties of the signal itself but rather the social conventions surrounding the signal (also known as “*conventional signals*”) (Hurd 1997, Enquist 1985, Searcy et al. 2008, Vehrencamp 2000). Here we find that stimuli with HI calls are more frequently attacked by receivers, which is consistent with a receiver retaliation rule.

Limits to Reliability

Although HI calls significantly predict subsequent attacks, the reliability of HI as an aggressive signal has its limits. 10.8% of the females that ultimately did not attack still used HI calls during the signaling period (termed “over-signaling” or “bluffing”). These females can be considered “dishonest” in the classic sense (Akçay et al. 2013). Intriguingly, 68.8% of attacks were not preceded by a HI call (termed “under-signaling”). These results are qualitatively similar to studies on sparrows that also find a higher rate of under-signaling than over-signaling (soft song in song sparrows: under = 40%, over = 23%, soft song in swamp sparrows: under = 44%, over = 3.2% (Searcy et al. 2013); song matching in song sparrows: under = 23%, over = 8.3% (Akçay et al. 2013)). Under-signalers in our experiment fell into two qualitatively different categories: those that responded immediately to playback with attacks (37.5% of attacks) and those that interacted with playback but ultimately did not signal their eventual attack (31.3% of attacks).

The limited reliability of HI as a signal has theoretical support. Game theory predicts that a certain level of bluffing through over-signaling can be stable (Gardner & Morris 1989, Adams & Mesterton-Gibbons 1995, Számadó 2008, Botero et al. 2010). Over-signaling can also result from assessment errors (Wiley 1994, Botero et al. 2010), intervening distractions (Searcy et al. 2006, Searcy et al. 2013), or the artificial, non-interactive nature of the simulated intruder

(Laidre & Vehrencamp 2008). Under-signaling is more difficult to explain, although it is predicted in some models (“Trojan sparrow” strategy: Hurd 1997) and has been detected in other experiments (Searcy et al. 2006, Ballentine et al. 2008, Searcy et al. 2013, Akçay et al. 2013). Theory disagrees on whether low or high fighting ability individuals should be more likely to under-signal (Christy & Rittschof 2010, Searcy et al. 2013). We could find no clear differences in size, condition, age, settlement date, reproductive success, or the number of days a female held a territory between honest signalers and under-signalers, although our sample sizes were small. However, a simulated female intruder at the center of a territory in close proximity to a nest with vulnerable eggs might be such an intense threat that some females, regardless of fighting ability, forego signaling altogether and go straight for attack. In fact, all females that attacked the set-up 30 seconds or less after playback started gave no vocalizations and thus fell into this under-signaling category (6 out of 11 under-signalers).

Low-amplitude Signaling

HI calls bear a striking similarity to soft songs in male songbirds, both in terms of acoustic structure and context. In addition to their characteristic low amplitude, soft songs are often characterized by high frequencies and fewer tonal elements than typical broadcast songs (Dabelsteen et al. 1998, Anderson et al. 2008, Xia et al. 2013, Reichard et al. 2013). HI calls share these characteristics. Like HI calls, male soft song is also found in both courtship as well as aggressive contexts in many species (Reichard & Anderson 2015, Reichard & Welklin 2015). Male house wrens also produce soft songs (“whisper song”) during the female’s fertile period as well as occasional territorial intrusions (Johnson & Kermott 1991). Finally, low-amplitude songs and calls are some of the few avian signals that has been shown unequivocally to be aggressive signals in a growing list of species (song sparrow: Searcy et al. 2006, Templeton et al. 2012;

corncrake: Ręk & Osiejuk 2011; black-throated blue warbler: Hof & Hazlett 2010; swamp sparrow: Ballentine et al. 2008; brownish flanked bush warbler: Xia et al. 2013).

In both courtship and close range aggressive interactions, intended receivers are in close proximity to senders. However, there is currently an open debate over why low-amplitude rather than high-amplitude signals are the most aggressive signal in many species, especially since the latter seems more intimidating (Searcy et al. 2006, Akçay et al. 2015, Reichard & Anderson 2015). Early work suggested males benefit from using low-amplitude signals that limit eavesdropping by predators and other conspecifics (Dabelsteen et al. 1998), although this is not the case in the most thoroughly studied empirical system, the song sparrow (Searcy & Nowicki 2006). Others have suggested that the short-range nature of low-amplitude sounds communicates a bird's willingness to take risks (Laidre & Vehrencamp 2008, Hof & Hazlett 2010), is an unambiguous signal of a sender's attention (Ballentine et al. 2008), or is merely the result of adopting a head-forward, attack-ready stance (Akçay et al. 2011). Despite which hypothesis wins out, males may be expected to face an opportunity cost to switching from high-amplitude to low-amplitude signaling by reducing their ability to communicate to potential mates or territorial intruders located at greater distances (Anderson et al. 2007, Searcy et al. 2008).

The limited role of female house wrens in territorial defense may help them avoid some of the trade-offs males encounter when choosing to use low-amplitude vocalizations. Like males of many songbirds, male house wrens broadcast extremely loud territorial songs that human observers can easily hear multiple territories away (Johnson & Kermott 1991). Over hundreds of hours of observation at our study site, we have never heard female house wrens sing such loud, consistent songs. Searcy et al. (2006) found that male song sparrows singing soft song faced more intrusions by neighboring birds, perhaps because the territorial bird appeared absent to

distant receivers. Therefore, males suffer an important cost when choosing to use soft song. Since female house wrens do not normally sing these loud advertising songs to defend territory boundaries, it seems unlikely they would face the same trade-off when using low-amplitude signals. Female house wrens may be expected to use predominantly short range vocalizations since they can transmit to intended receivers- mates and intruders threatening local resources such as the nestbox- while limiting energy expenditure and reducing conspicuousness to predators. Low-amplitude signals of aggressive intent could be more common than appreciated in other females taking similar roles.

Conclusion

Much of the empirical work on vocal signaling has focused male signals used during sexual competition. As a general rule, female vocalizations are understudied in most groups (Langmore 1998, Riebel et al. 2005, Garamszegi et al. 2007). Furthermore, most of the early work on vocal signaling focused on high-amplitude signaling, likely because low-amplitude signals are more likely to escape notice. Here we demonstrate that an easily overlooked signal, a low-amplitude female call, is an aggressive signal used during female-female house wren conflicts. Our data suggest that the reliability of this call is likely maintained by a receiver retaliation rule. To our knowledge this is the first low-amplitude aggressive signal that has been reported in female birds. We suggest that the nature of female competition in many species may actually make low-amplitude signaling a particularly common signaling form in females. Males and females share many challenges and constraints during aggressive contests. However, female biology may shape female conflicts in ways unique to females. Therefore, future investigations into female communication systems may provide a fruitful opportunity to test how and why particular signals evolve.

CHAPTER 2 ACKNOWLEDGEMENTS

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APPENDIX

APPENDIX

Supplemental figures for Chapter 2: song analysis

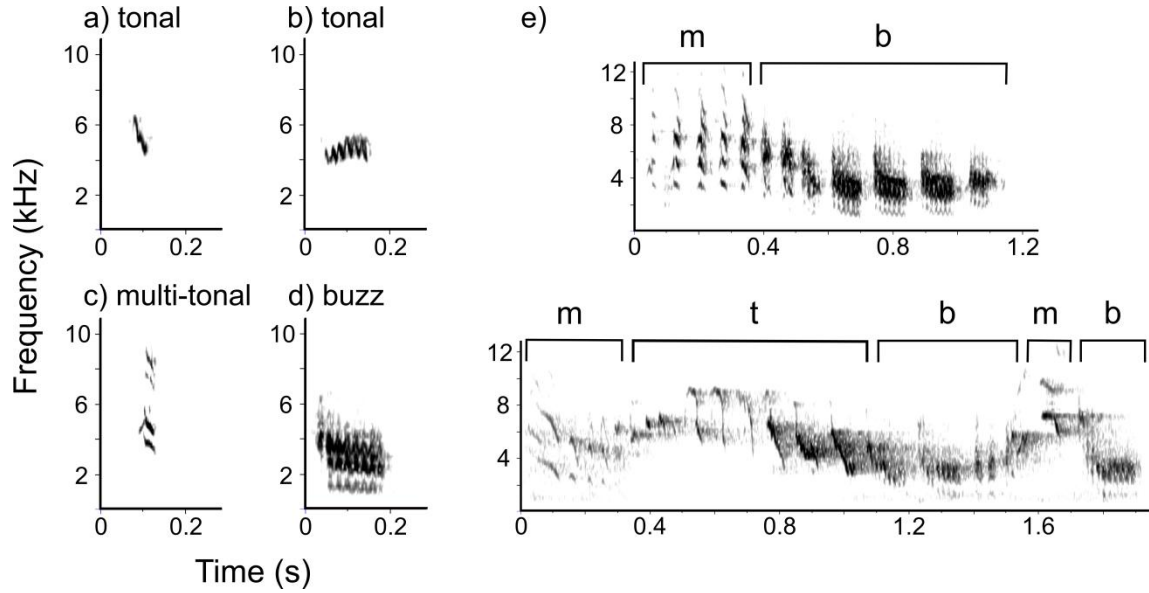


Figure 2.8: Examples of tonal (a), (b), multi-tonal (c), and buzz (d) syllables from female house wren songs. (E) shows two female house wren songs diagrammed by syllable types (t = tonal, m = multi-tonal, b = buzz).

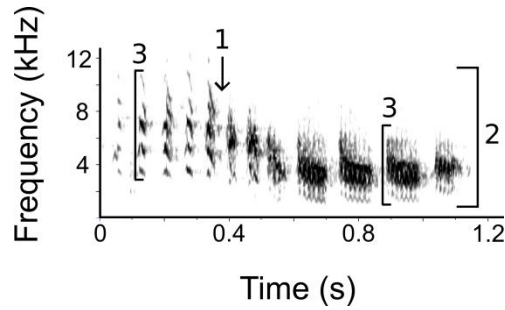


Figure 2.9: (1) A switch between two syllable types. (2) The frequency spread of the song. (3) The frequency spread of two song syllables. We take the standard deviation of the frequency spread of all the syllables in the song to capture variation in song shape.

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CHAPTER THREE

Female house wrens value access to nest cavities more than
benefits from males during conflicts with female intruders

Cara A. Krieg & Thomas Getty

ABSTRACT

Individuals should fight hardest to win the most valuable resources. Whereas males frequently compete for fertile females, females more often compete for particular quality males, male care, or resources required to breed. We asked whether female house wrens (*Troglodytes aedon*) place more value on the benefits offered by males or access to the nesting cavity during aggressive conflicts with simulated female intruders. We randomly assigned house wren pairs to receive zero or two supplemental nestboxes. We then assayed female aggression. Intruders on three-box territories may settle as a secondary female. Therefore, the risk of losing the cavity and territory is highest on a one-box territory, and the risk of polygyny is highest on a three-box territory. If direct or indirect benefits offered by males are most valuable to females, we predicted that three-box females would be most aggressive. If access to a nesting cavity is most valuable to females, we predicted that one-box females would be most aggressive. We found that one-box females were significantly more aggressive. Aggressive behavior is likely adaptive, as females that switched territories between broods in 2012 were significantly more likely to suffer complete clutch failure than females that retained the same territory. Altogether, these results suggest that intrasexual competition for access to nesting cavities may be an important component of female fitness in house wrens. This work also contributes to a growing body of evidence that females often compete for resources required to breed.

INTRODUCTION

How aggressively an individual responds during a fight often depends on how motivated that individual is to win (Enquist & Leimar 1987, Leimar & Enquist 1984, Maynard Smith & Parker 1976, Parker 1974). Fatal fights should only evolve when the value of the contested resource is very high for both contestants (Enquist & Leimar 1990, Grafen 1987). When asymmetries in motivation exist, they can sometimes be even more predictive than asymmetries in size and strength in determining the ultimate outcome of the fight (Dale & Slagsvold 1995, Dugatkin & Ohlsen 1990, Elias et al. 2010, Jonart et al. 2007, Tibbetts 2008, Yovel 1989). Therefore, game theory suggests that the intensity of fights can be used to determine which resources are the most valuable to the animals involved. Fights should be particularly common between members of the same sex, since they are most likely to require the same resources to maximize fitness (Stockley & Campbell 2013).

Intrasexual aggression between females has recently attracted widespread attention (Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, Rosvall 2011, Rubenstein 2012, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012). Much of the work on aggression in males has focused on intrasexual competition for mates. From this perspective, however, is it unclear why females of most species would fight one another. Nevertheless, intrasexual aggression among females is common across taxa and mating systems (Clutton-Brock 2009, Rosvall 2011, Stockley & Campbell 2013). In fact, in some cases female aggression is even more intense than male aggression (Archard & Braithwaite 2011, Ayer & Whitsett 1980, Clutton-Brock & Vincent 1991, Elias et al. 2010, Murphy et al. 2009, Papadopoulos et al. 2009, Petrie 1983; French et al. 2013). What the field needs now is a

broad understanding of why females in different species compete with an explicit focus on resources that should be important for female fitness (Cain & Rosvall 2014, Rubenstein 2012).

Biologists studying intrasexual aggression between females have focused on two major reasons why females might compete: competition for benefits offered by males and competition for other breeding resources (Clutton-Brock 2009, Rosvall 2011). Benefits offered by male mates frequently affect female fitness in both the short-term (e. g. male provisioning) and the long-term (e.g. indirect genetic benefits) (Andersson 1994, Andersson & Simmons 2006, Jones & Ratterman 2009). As a result, females sometimes compete aggressively with other females to gain or keep exclusive access to males offering direct (Ekstrom et al. 2007, Gwynne & Simmons 1990, Liker & Székely 1997, Sandell 1998, Summers 1989, Veiga 1992, Wong et al. 2008) and indirect benefits (Bro-Jørgensen 2002, Rillich et al. 2009, Sæther et al. 2001). Additionally, female fitness can be strongly influenced by resources unrelated to mates, such as food, future resources for offspring, or resources required to initiate breeding attempts (e.g nest sites, oviposition sites, rank in cooperative breeding species). Females often compete aggressively for these resources as well (arachnids: Elias et al. 2010; birds: Griggio et al. 2010, Heinsohn & Legge 2003, Heinsohn et al. 2005, Renison et al. 2003, Robinson 1985, Rosvall 2008; carnivores: Clutton-Brock et al. 2006, Frank 1986; fish: Almada et al. 1995, Borg et al. 2002, insects: Eggert et al. 2008, Shelly 1999, Watson & Simmons 2010; primates: Hohmann & Fruth 2003, Pusey & Schroepfer-Walker 2013; rodents: Young & Bennett 2013; ungulates: Robinson & Kruuk 2007, reviewed in Bro-Jørgensen 2011). Few studies have directly compared these two motivations for female fights.

Game theory predicts individuals should fight hardest to win resources that are the most valuable (Enquist & Leimar 1990, Grafen 1987). We use this logic to ask which is more

important to female house wrens (*Troglodytes aedon*): benefits offered by males, or access to a nesting cavity. House wrens are facultatively polygynous secondary cavity nesters with biparental care (Kendeigh 1941), meaning both benefits offered by males and cavity access should influence female fitness. Male house wrens provision nestlings and defend against predators (Johnson et al. 1993, Johnson & Albrecht 1993). Removing males during the nestling period dramatically reduces female fitness (Johnson et al. 1992). Males may sometimes also be polygynous (5-45% of males; Drilling & Thompson 1991; Johnson & Kermott 1991a, 1991b; Kendeigh 1941), making intruding females a potential threat to future male assistance. Nest cavities are an extremely important resource as well. Each female needs a cavity in order to breed, but they cannot create their own. Cavity availability often limits the population sizes of cavity nesting birds (Newton 1994). However, it is possible that once house wrens begin breeding, males take over cavity defense, and thus maintaining exclusive access to benefits offered by mates becomes most important for females.

To determine whether benefits offered by males or access to a nest cavity are more important drivers of intrasexual aggression for female house wrens, we (1) randomly assigned each pair to receive zero or two supplemental nestboxes after courtship was complete and (2) then assayed the female's aggressive response to a simulated female intruder. If an intruder wins on a three-box territory, the original female may either be forced to accept a secondary female on the territory or be forced to find a different territory. The former option does not exist on one-box territories. Therefore, one-box females face a greater risk of losing the nesting cavity and territory, whereas three-box females face a greater risk of polygyny. Additionally, earlier work in this and other populations suggests that females behave as though males randomly assigned supplemental nest cavities are higher quality mates than males with a single cavity (Dubois et al.

2006, Eckerle & Thompson 2006, Grana et al. 2012). Therefore, if the benefits offered by males are most important to females, three-box females should be most aggressive. Alternatively, if access to a cavity is most important to females, one-box females should be most aggressive.

METHODS

Study Site and Study System

This study was conducted in 2012 and 2013 with a house wren population nesting at the Lux Arbor Reserve at the Kellogg Biological Station in Barry County, MI (42° 29' N, 85° 28' W). This 529 hectare protected area contains forest, grassland, and fragmented agricultural fields. For the last 15 years, we have provided nestboxes in the same locations on suitable edge habitat (Dubois & Getty 2003). Each house wren territory contained a cluster of 3 nestboxes separated by 5-10 m. Each box cluster was at least 100 m from the next cluster, making it easy for a single male to defend one cluster but difficult to defend more than one. Overall the population contains 59 house wren territories centered around these box clusters.

The population contained 56 females in 2012 and 71 females in 2013. Each bird was trapped on the nest or captured in a mist net. We sometimes used playback of male or female song as mist net lures. We monitored these nets continuously and no birds suffered adverse effects. If we captured birds prior to aggression testing, at least 3 days separated capture and testing. Each bird was given a USFWS band and three color bands to allow for individual identification. We measured female mass to the nearest 0.1 g and tarsus to the nearest 0.01 mm. To measure female condition, we used the residuals from a regression of tarsus on mass with nesting stage included as a covariate to account of systematic differences in female mass between incubation and non-incubation stages (for similar approach, see DeMory et al. 2010).

Box Number Manipulation

In 2012 and 2013, we assigned each mating pair to the one-box or three-box treatment. Only one box was initially open on each territory. Once females began lining the nest with soft feathers in preparation for egg laying, we randomly assigned box treatment in an alternating fashion. For three-box territories, we removed the corks from the entrances of the second and third boxes. Treatments were approximately balanced in each year (2012: 21 one-box, 21 three-box; 2013: 21 one-box, 19 three-box).

When male house wrens advertise for a mate, they sing conspicuously and attempt to lead the female to the entrance of the box (personal obs., Johnson & Kermott 1991b). We can use this conspicuous male behavior to detect natural cavities. Despite extensive searching in previous years, we knew of only one territory that contained natural cavities used by house wrens. This may be due to the relative scarcity of old trees in the forests at our study site. In all years, house wrens on this territory chose to use the nestbox instead of the natural cavity. We assigned the three-box treatment to this territory in both years. Thus, territories assigned to the one box treatment likely contain only one suitable cavity.

Aggression Trials

We assayed the aggressiveness of females by measuring their responses to playback of simulated female intruders. We placed a speaker and a wooden model house wren within 1.5 m of the active nestbox. After 5 minutes of silence (pre-playback period), females heard 5 minutes of female house wren song (playback period) followed by an additional 5 minutes of silence (post-playback period). We recorded all vocalizations and narrated all movements using a Marantz PMD 660 solid state digital recorder attached to a Sennheiser ME62 omnidirectional microphone and a Mineroff parabolic reflector. Each female heard a randomly selected playback

track from the set being used that year (2012: 6 stimuli, 2013: 15 stimuli). Each playback stimulus contained 6 high quality song recordings from a single individual, each separated by 5 second of silence, filtered at 1 kHz, standardized to a peak amplitude of 1.4 kU using Raven Pro v1.4 (Bioacoustics Research Program 2011), and played back at an amplitude of 70-75 dB measured at 1 m by an Extech digital sound level meter (model 407732). We never tested females with their own song, songs of an immediate neighbor, or playback they heard in a previous year. As part of another study, females also heard a heterospecific song trial and a male song trial. We randomized trial order and conducted only one trial per day. Exposure to previous house wren playback did not alter aggressive responses of females (gamma generalized linear model; order: $\chi^2 = 2.287$, $P = 0.13$; trial type by order interaction: $\chi^2 = 1.64$, $P = 0.2$). We tested all females between sunrise and 12:30 EST within the first four days of the female's egg laying period. For additional details on the playback experiment see Chapter 1, "*Playback Experiment*" and Chapter 2, "*Playback Stimuli*" and "*Playback Experiment*".

We assigned each female an aggression score based on four behaviors that are commonly used as proxies for aggressiveness in playback studies: attacks, dives, latency to approach the speaker within 5 m, and the difference in spent within 1 m of the tripod and speaker during the playback period compared to the pre-playback period. We defined dives as swooping rapidly within several centimeters of the model or the speaker. Attacks required actual physical contact. We calculated scores using the formula below. Any female that was not seen or heard during the entire 15 minute aggression trial was dropped from subsequent analyses. For more details on aggression scores see Chapter 2, "*Aggression Score*".

Aggression score = $3 * (\# \text{ of attacks}) + 2 * (\# \text{ of dives}) + 1 * ((300 - \text{seconds to approach within 5m})/300) + 1 * ((\text{seconds within 1m during playback} - \text{seconds within 1m during pre-play})/300)$

Box Treatment Analysis

To determine whether box treatment affected the aggressiveness of females, we used a generalized linear model with a gamma distribution. Gamma generalized linear models are suitable for continuous data with right skewed errors (Bolker 2008). Models for both the 2012 and 2013 included box treatment and female mass as covariates. Models using female condition instead of mass produced the same results (see Appendix A for more details). In the 2012 model, we included playback stimulus as a main effect since it had too few levels to be considered a random effect (levels = 6). In the 2013 model, playback stimulus was included as a random effect (levels = 15). We have previously shown that playback stimulus significantly affects the aggressive response of females (see Chapter 2, Figure 2.4). Because we dropped females from our analyses if they did not respond during the aggression trial, this analysis had 21 one-box and 20 three-box females in 2012 and 16 one-box and 17 three-box females in 2013.

Fitness Consequences of Switching Territories

We investigated whether switching territories between broods within a year affected female reproductive success. Because the eviction experiment carried out in 2013 interfered with both territory switching and accurate calculations of reproductive success, we only considered 2012 in this analysis (see Chapter 4, “*Eviction Experiment*” for details). We used a generalized linear model with a binomial distribution to determine whether females were more likely to suffer total clutch failure after switching territories. To avoid pseudo-replication, we

only included each female's second brood, even if females attempted three. This analysis contained 15 females that switched and 13 females that remained on the same territory.

Statistical Note

We conducted all statistical tests in R version 3.0.2 (R Core Team 2013; package 'car': Fox & Weisberg 2011; package 'lme4': Bates et al. 2014; package 'bbmle': Bolker & R Development Core Team 2014; package 'AICcmodavg': Mazerolle 2015). We assessed all models for heteroscedasticity and normality of residuals using model diagnostic plots and outliers using Cook's distance. We used VIF values to assess multicollinearity (cutoff VIF value of 4, all VIF values were below 2). Because generalized linear models do not produce the R^2 values used in traditional linear models, we instead calculated goodness-of-fit with pseudo- R^2 values based on a likelihood ratio test (Magee 1990) using the "r.squaredLR" command in the package "MuMIn" (Barton 2015).

Ethical Note

This research was approved by the Michigan State University's Institutional Animal Care and Use (ACUC) panel. Field work was conducted under appropriate federal and state permits (MSU IACUC Animal Use Form numbers 04/11/076-00, 03/14-062-00; U. S. Fish and Wildlife Service Federal Banding Permit number 23587; Michigan Department of Natural Resources Scientific Collectors Permit number SC 1432, U. S. Fish and Wildlife Service Permit number MB01550B-1). No females abandoned territories following our tests. No birds suffered adverse effects during capture and measurement.

RESULTS

Females assigned to the one-box treatment responded significantly more aggressively than three-box females during simulations of intruding females (Figure 3.1; 2012: $\chi^2 = 8.675$, $P = 0.003$, model LR pseudo- $R^2 = 0.57$; 2013: $\chi^2 = 4.356$, $P = 0.036$, model LR pseudo- $R^2 = 0.20$). One-box females were more aggressive in both 2012 and 2013, although females were overall more aggressive during 2012. When we replaced the female's aggression score with the number of dives and attacks, the results were qualitatively similar for 2012. Not enough birds dove or attacked in 2013 to test for a difference (see Appendix A for details). Aggression was the only characteristic that differed between treatments. Female condition, mass, mate condition, mate mass, and male feeds per offspring did not differ between box treatment (t-tests; condition: $t = 0.27$, $P = 0.79$; mass: $t = 0.40$, $P = 0.69$; mate condition: $t = 0.17$, $P = 0.87$; mate mass: $t = 0.21$, $P = 0.83$; male provisioning: $t = -0.39$, $P = 0.70$).

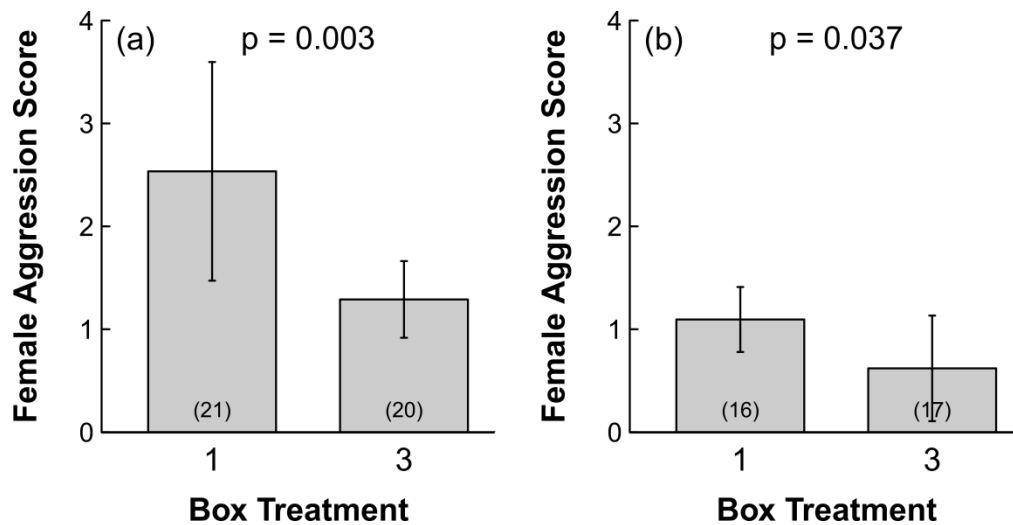


Figure 3.1: The effect of box treatment on the aggression score of females during simulated female intrusions ($\pm$ SE) in 2012 (a) and 2013 (b). P -values indicate results from a gamma generalized linear model (a) and mixed model (b) after accounting for the effects of female mass and playback stimulus. Numbers in parentheses indicate the number of birds tested.

Table 3.1: Results from a generalized linear model with a gamma distribution (2012) and generalized linear mixed model with a gamma distribution (2013) on the effect of box treatment and female mass on female aggression. Bolded values indicate $P < 0.05$.

2012			
Variable	Estimate $\pm$ SE	χ^2	P
Box treatment	0.077 $\pm$ 0.027	8.675	0.003
Female mass	0.002 $\pm$ 0.017	0.013	0.908
Playback stimulus		36.35	<0.001
2013			
Box treatment	0.089 $\pm$ 0.043	4.356	0.037
Female mass	0.018 $\pm$ 0.020	0.825	0.364
Playback stimulus (rand effect)	-	-	-

Bigger females were not more aggressive. Female mass was not a significant predictor of aggression score in either the 2012 or 2013 model (Table 3.1). Results are similar when we use female condition instead of female mass (see Appendix B for details).

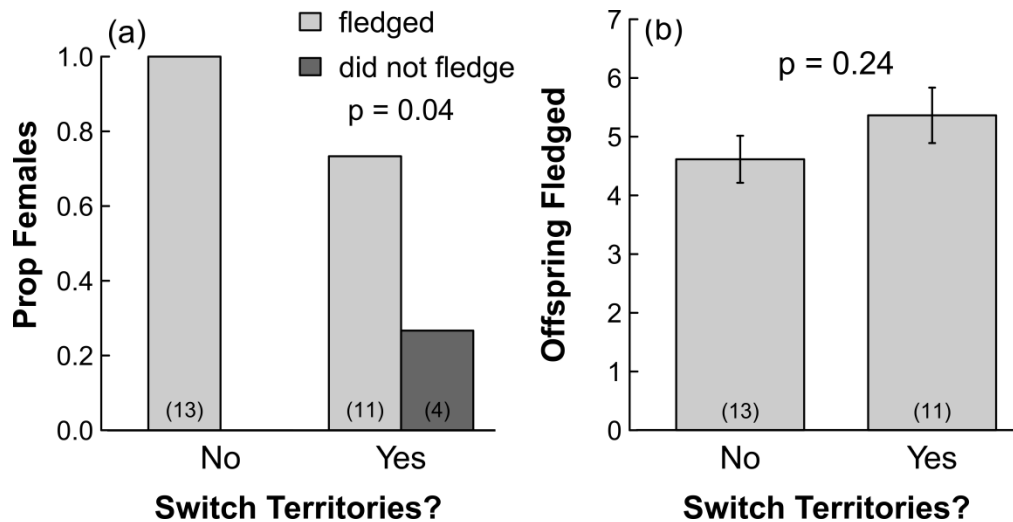


Figure 3.2: (a) Females that switched territories between their first and second brood were significantly more likely to fail to fledge any second brood offspring (binomial generalized linear model, $P = 0.036$). (b) When females that failed are removed, the mean ($\pm$ SE) number of offspring fledged did not differ between females that stayed and switched (Mann-Whitney test, $P = 0.24$). Numbers in parentheses indicate the number of females in each category.

Females that switched territories for their second brood in 2012 were more likely to suffer complete clutch failure (Figure 3.2a; binomial generalized linear model: $\chi^2 = 4.39$, $P = 0.036$). In fact, the only females that failed completely were females that switched. If we eliminate complete failure females, females in each category produced approximately the same number of offspring (Figure 3.2b; Mann-Whitney test: $W = 51$, $P = 0.24$). Females that stayed on the same territory did not necessarily retain the same mate. 15% of females that stayed on their territories mated with a new male in the second brood. Similarly, 11% of females that switched territories retained the mate from their first brood.

DISCUSSION

Female house wrens were most aggressive toward female intruders when the threat to losing the nest cavity and territory was the greatest. Therefore, females appear to value continued access to the cavity more highly than benefits offered by their mates. Female house wrens assigned to the one-box treatment were significantly more aggressive in both 2012 and 2013 than females provided with 3 nestboxes. Although females with 3 boxes could possibly share the territory if they lose to a female opponent, females with a single box would be forced to switch territories. Female house wrens do not appear to adjust their aggressiveness to retain exclusive access to benefits provided by their mate. Previous work in this population has shown that females behave as though males assigned to three-box territories are higher quality (Dubois et al. 2006). Additionally, polygyny can only occur on three-box territories. However, three-box females were actually less aggressive than one-box females. These results suggest that access to a cavity is more valuable than access to the benefits provided by the male at this stage in the

season. Competition for valuable nesting cavities may be a primary driver behind same-sex aggression among female house wrens.

This behavior is likely adaptive, as females that switch territories between their first and second broods were more likely to suffer complete clutch failure in their second brood. This difference in reproductive success has several possible causes. First, switching females may be more likely to move on to previously unoccupied lower quality territories where food resources may be more limited or nest cavities may be more vulnerable to predation. Second, females moving on to a new territory may be less familiar with the local resources than females that continue to breed in the same location. Finally, if females must compete for a new second-brood territory, they may have less energy remaining to then invest in reproduction (Fitzpatrick et al. 1995). Females that switched did not have a longer intraclutch interval or initiate clutches later in the season. Although some females may choose to switch to a higher quality territory or better mate, the decline in reproductive success for switching birds suggests a portion are forced to switch territories as a result of either predation or interference from other females.

Larger or better condition females were not more aggressive. These results are consistent with other studies finding that absolute or relative differences in size, strength, or condition do not predict the aggressiveness of females or female fight outcomes (Arnott & Elwood 2009, Dale & Slagsvold 1995, Draud et al. 2004, Elias et al. 2010, Koivula et al. 1993, Sinn et al. 2008, While et al. 2009). Intriguingly, a handful of studies that have compared male and female fights in the same species have found that physical characteristics like size predict outcomes of male but not female fights (Draud et al. 2004, Elias et al. 2010, Koivula et al. 1993, Robinson 1985). Instead, asymmetries in motivation were more important in these female contests. This sex-difference in fighting rules may be explained by sex-differences in contested resources. Females

more frequently compete for breeding resources that are only valuable only during short, discrete stages of the female's breeding cycle. Therefore, flexible asymmetries in motivation rather than more fixed asymmetries in size and strength may be more important in determining the outcome of female than male fights in an array of species (Draud et al. 2004).

Male quality should influence female fitness, both through nestling care and genetic qualities passed on to offspring. However, we found no evidence that females are more aggressive in defending their mating status when they perceive their mates as higher quality. Studies in other house wren populations have found that males randomly given extra cavities prior to female arrival attract females faster than males without supplemental cavities (Eckerle & Thompson 2006, Grana et al. 2012). A previous study in our study population found that females with mates randomly assigned supplemental boxes produced larger clutches with a higher percentage of male offspring than males with a single box. This result suggests females perceived these males as higher quality and invested in producing sons that could inherit these successful characteristics (Dubois et al. 2006). Under natural conditions, a male that is able to defend multiple cavities likely has characteristics that make him successful in intrasexual competition (DeMory et al. 2010, Duckworth 2006). Our results suggest that, regardless of perceived mate quality, females react most strongly to the risk of losing a territory once they have begun breeding. The finding that one box females are more aggressive than three box females also suggests that the risk of losing the cavity and territory is more important than the risk of polygyny, as the probability of polygyny is zero on a one box territory. Taken together, these results imply that competition for nesting cavities is an important driver of intrasexual aggression in house wrens and likely an important influence on female fitness.

Work with other polygynous species has found that intrasexual aggression plays an important role in maintaining monogamy (Liker & Székely 1997, Sandell 1998, Slagsvold & Lifjeld 1994, Veiga 1992, Wong et al. 2008). In contrast to this study, work with European starlings (*Sturnus vulgaris*) found that females mated to males with supplemental boxes were more aggressive toward caged female intruders than females mated to males with a single box (Sandell & Smith 1997). While monogamy is likely the ideal situation for female house wrens, our results suggest that the risk of completely losing a territory altogether is a far greater factor in female aggression. Consistent with this finding, work in other populations of house wrens suggests that primary females suffer relatively minor costs to polygyny (Czapka & Johnson 2000).

Males often fight over females. However, females often fight over resources unrelated to males (Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, Rosvall 2011). Here we show that female house wrens adjust their aggressiveness towards other females based on the risk of losing their nesting cavity and territory but not based on the perceived quality of their mates or the potential for polygyny. Aggressive, sometimes intensely violent, competition for nest sites is common among other cavity nesting birds as well (tree swallow (*Tachycineta bicolor*): Chek & Robertson 1991, Dunn & Hannon 1991, Leffelaar & Robertson 1985, Rosvall 2008); pied flycatcher (*Ficedula hypoleuca*): Dale & Slagsvold 1995; Eastern bluebird (*Sialia sialis*): Gowaty 1981, Gowaty & Wagner 1988; eclectus parrot (*Eclectus roratus*): Heinsohn & Legge 2003, Heinsohn 2008, Heinsohn et al. 2005). Across the animal kingdom, females are known to fight other females for nest sites (blennioid fish (*Salaria pavo*): Almada et al. 1995; common goby (*Pomatoschistus microps*): Borg et al. 2002; Galápagos marine iguanas (*Amblyrhynchus cristatus*): Rubenstein & Wikelski 2005), oviposition sites

(jumping spider (*Phidippus darus*): Elias et al. 2010; oriental fruit fly (*Bactrocera dorsalis*): Shelly 1999) , food for themselves or offspring (rock sparrow (*Petronia petronia*): Griggio et al. 2010; burying beetles (*Nicrophorus vespilloides*): Eggert et al. 2008; dung beetle (*Onthophagus sagittarius*): Watson & Simmons 2010; chimpanzees (*Pan troglodytes*): Pusey & Schroepfer-Walker 2013; Soay sheep (*Ovis aries*): Robinson & Kruuk 2007), and social rank (meerkats (*Suricata suricatta*): Clutton-Brock et al. 2006; Damaraland mole-rat (*Fukomys damarensis*): Young & Bennett 2013). Understanding why and how females fight requires understanding what is most “worth fighting for”. For females, that is not always a male.

CHAPTER 3 ACKNOWLEDGEMENTS

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APPENDICES

APPENDIX A

Chapter 3 aggression models using dives and attacks as a dependent variable

A model using the number of times a female dove at or attacked the simulated intruder instead of aggression score produced qualitatively similar results in 2012 (Figure 3.3, Table 3.2). Here we used a generalized linear model with a Poisson distribution, which is appropriate for count data. In 2013, only three birds in this data set dove or attacked. In the three-box treatment, one bird dove 3 times. In the one-box treatment, one bird dove twice and another dove once.

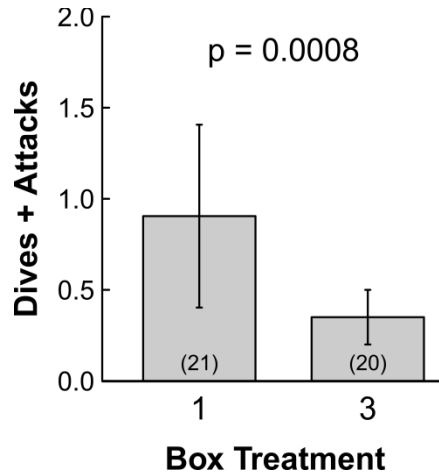


Figure 3.3: The mean number of dives and attacks ($\pm$ SE) females made during a simulation of a female intruder based on box treatment. Numbers indicate the number of females tested.

Table 3.2: The results from a generalized linear model with a Poisson distribution on the effect of box treatment, female mass, and playback stimulus on the number of dives and attacks during playback (LR pseudo- $R^2 = 0.80$). Bolded values indicate $P < 0.05$.

Variable	Estimate $\pm$ SE	χ^2	<i>P</i>
Box treatment	-1.478 $\pm$ 0.471	11.267	0.0008
Female mass	-0.021 $\pm$ 0.280	0.005	0.942
Playback stimulus		45.904	< 0.001

APPENDIX B

Chapter 3 aggression models using female condition instead of female mass

We did not include both female mass and female condition in the Chapter 3 aggression models because these variables were highly correlated (Pearson's correlation coefficient: 0.92). Replacing female mass with female condition does not change the overall conclusions. Females that are bigger or in better condition are not more aggressive. Below are the results from the models with mass and with condition for 2012 and 2013 (Tables 3.3-3.6). Combining both models using model-averaged parameter estimates based on relative AICc values yields the same results as presented in the main text of Chapter 3 (Table 3.7, 3.8; Buckland et al. 1997, Burnham & Anderson 2002).

Table 3.3: The original 2012 aggression model using female mass as a covariate (LR pseudo- $R^2 = 0.57$). Bolded values indicate $P < 0.05$

Variable	Estimate $\pm$ SE	χ^2	P
Box treatment	0.077 $\pm$ 0.027	8.675	0.003
Female mass	0.002 $\pm$ 0.017	0.013	0.908
Playback stimulus		36.35	< 0.001

Table 3.4: An alternate 2012 aggression model using female condition as a covariate (LR pseudo- $R^2 = 0.58$). Bolded values indicate $P < 0.05$.

Variable	Estimate $\pm$ SE	χ^2	P
Box treatment	0.072 $\pm$ 0.026	7.760	0.005
Female condition	-0.014 $\pm$ 0.020	0.490	0.484
Playback stimulus		33.92	< 0.001

Table 3.5: The original 2013 aggression model using female mass as a covariate (LR pseudo- $R^2 = 0.20$). Bolded values indicate $P < 0.05$.

Variable	Estimate $\pm$ SE	χ^2	P
Box treatment	0.089 $\pm$ 0.043	4.356	0.037
Female mass	0.018 $\pm$ 0.020	0.825	0.364
Playback stimulus (rand effect)	-	-	-

Table 3.6: An alternate 2013 aggression model using female condition as a covariate (LR pseudo- $R^2 = 0.18$). Bolded values indicate $P < 0.05$.

Variable	Estimate $\pm$ SE	χ^2	P
Box treatment	0.085 $\pm$ 0.044	3.689	0.055
Female condition	0.004 $\pm$ 0.022	0.037	0.847
Playback stimulus (rand effect)	-	-	-

Table 3.7: Model averaged coefficients combining the original and alternate 2012 aggression models. Bolded values indicate confidence intervals that do not overlap 0.

Variable	Model averaged estimates	95% unconditional CI
Box treatment	0.07	0.02 – 0.13
Female mass	0.02	-0.03 – 0.04
Female condition	-0.01	-0.05 – 0.03

Table 3.8: Model averaged coefficients combining the original and alternate 2013 aggression models. Bolded values indicate confidence intervals that do not overlap 0.

Variable	Model averaged estimates	95% unconditional CI
Box treatment	0.09	0 – 0.17
Female mass	0.02	-0.02 – 0.06
Female condition	0	-0.04 – 0.05

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CHAPTER FOUR

Aggressive female house wrens are more successful during experimentally enhanced competition: a population-wide approach

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ABSTRACT

Male animals often compete aggressively to gain sexual access to females. Researchers studying intrasexual aggression among females have suggested that females are more likely to compete aggressively for access to resources critical to reproduction than for access to mates. Here we examined whether female-female aggression is important during periods of intense resource competition by limiting the number of available nesting cavities in a population of house wrens (*Troglodytes aedon*). We assayed female aggressiveness in response to a simulated female intruder and then experimentally elevated the number of floating conspecifics by evicting a subset of birds. Females that were more aggressive towards simulated female intruders than their opponents were significantly more likely to win contests over cavities. Aggression also helped females cope with elevated resource competition. This experimental manipulation dramatically increased the number of eggs destroyed by intruding house wrens. Females that were aggressive toward simulated female intruders lost significantly fewer eggs to ovide committed by conspecifics. House wrens may be another example in a growing list of species in which intrasexual aggression among females is under selection through competition over critical breeding resources.

INTRODUCTION

Variation in female fitness is thought to be driven largely by variation in female fecundity and offspring care, rather than variation in access to mates (Bateman 1948, Shuster & Wade 2003, Trivers 1972). Theory predicts that investing energy in traits used in competition for mates reduces energy that could potentially be invested in reproduction (Chenoweth et al. 2006, Fitzpatrick et al. 1995). Thus, investment in competition should lower female fitness. For this reason, intrasexual aggression, ornaments, and armaments in females have historically been considered merely correlated responses to selection acting on males in the majority of mating systems (Amundsen 2000, Lande 1980).

However, there is now growing recognition that competition for critical breeding resources strongly influences female fitness. In many species across a wide range of taxa, female compete with other females for access to resources required to breed at all (e.g. nesting sites) or resources that strongly influence fitness after breeding commences (e.g. food resources, paternal care) (Berglund et al. 1993, Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, LeBas 2006, Pusey & Schroepfer-Walker 2013, Rosvall 2011a, Rubenstein 2012, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012). This competition leads to the evolution of behaviors, ornaments, and armaments used in intrasexual female aggression in a process that is in some respects analogous to sexual selection in males (Clutton-Brock 2007, Clutton-Brock 2009, Lyon & Montgomerie 2012, Rosvall 2011a, Tobias et al. 2012, West-Eberhard 1983).

Here we investigate whether female aggression is a trait used in intrasexual competition among female house wrens. House wrens are small (10-12 g), sexually-monomorphic songbirds that breed throughout the Western hemisphere (Kendeigh 1941). House wrens are facultatively

polygynous and males provision nestlings during both broods during the 4 month breeding season. To breed at all, however, females must have access to a nesting cavity. House wrens are obligate secondary cavity nesters that are not known to egg dump. Both males and females will sometimes evict the same-sex member of an existing breeding pair, resulting in complete destruction of the original clutch (Belles-Isles & Picman 1986, personal observation). Thus, obtaining and maintaining possession of a nesting cavity is critical to reproductive success in female house wrens. Previous work in our study population showed that females reacted most aggressively toward simulated female intruders when they had the greatest risk of losing their nesting cavity (see Chapter 3). These results suggest that competition for cavities might be an important driver of intrasexual aggression among females in this species.

To determine whether intrasexual aggression helps females compete for access to nesting cavities, we manipulated competition on a population-wide scale by experimentally elevating the number of floating conspecifics. After assaying female aggressiveness using simulated female intruders, we then evicted a subset of birds from their nesting cavities and asked whether aggression helped females in this new competitive environment. We predicted that more aggressive females would be better at gaining new cavities and protecting existing cavities from floating conspecifics.

METHODS

Study Site and Study System

This study was conducted in 2013 with a house wren population nesting at the Lux Arbor Reserve at the Kellogg Biological Station in Barry County, MI (42° 29' N, 85° 28' W). This 529 hectare protected area contains forest, grassland, and fragmented agricultural fields. For the last 15 years, nestboxes have been provided in the same locations on suitable edge habitat (Dubois &

Getty 2003). Each house wren territory contained a cluster of 3 nestboxes located within 5-10 m of one another. Each box cluster was separated by at least 100 m from the next cluster, allowing the population to contain 59 house wren territories. Because male house wrens are conspicuous when advertising for females, it is easy to detect natural nesting sites. We are aware of only one territory with additional cavities, likely due to the relative scarcity of old trees in the forests at the study site. In this instance, the resident wrens still chose to nest in the nestbox rather than the natural cavity. This pair was not assigned to the eviction treatment (see “*Eviction Experiment*” below).

The population contained 107 breeding adults in 2012 (56 females), 124 adults in 2013 (71 females), and 66 adults in 2014 (33 females). We trapped each bird on the nest or in a mist net that sometimes used recordings of males or females as mist net lures. Mist nets were monitored continuously and no birds suffered adverse effects. If we captured birds before aggression testing, at least 3 days separated capture and testing. Each bird was given a USFWS band and three color bands to allow for individual identification. We measured female mass to the nearest 0.1 g and tarsus to the nearest 0.01 mm. We assessed condition by taking the residuals from a regression of tarsus on to mass for each sex with nesting stage included as a covariate in the female model to account of systematic difference in female mass between incubation and non-incubation stages (for similar approach see DeMory et al. 2010).

Aggression Trials

We tested each pair with a female playback trial and a male playback trial to measure their aggressiveness towards conspecifics. Each pair also heard a trial composed of heterospecific songs to determine each bird’s general response to human disturbance and playback near the nest (*Turdus migratorius*, *Dumetella carolinensis*, *Pipilo erythrophthalmus*,

Melospiza melodia, *Spizella passerina*, and *Spizella pusilla*). In each conspecific trial, we placed a speaker and a wooden model house wren within 1.5 m of the active nestbox. We were concerned that having a model present during the pre-playback period of the female but not heterospecific trials might alter responses. Therefore, in 2014, we replaced the bird model with a wooden egg of the same size and color that was presented during all trials. Aggressive responses to heterospecific and same-sex trials did not vary significantly based on model type (Mann-Whitney tests; female to heterospecific trial: $N = 112$, $W = 934.5$, $P = 0.69$; female to female trial: $N = 123$, $W = 1295$, $P = 0.69$; male to heterospecific trial: $N = 108$, $W = 1012.5$, $P = 0.86$; male to male trial: $N = 112$, $W = 1151$, $P = 0.66$). Data from the egg model year (2014) were only included in the analysis of territory takeovers (see details below). Because this analysis only compares data within each year, any undetected differences produced by model type should not alter the conclusions of this analysis.

Each trial followed the same procedure. After 5 minutes of silence (pre-playback period), birds heard 5 minutes of playback sounds (playback period) followed by an additional 5 minutes of silence (post-playback period). An observer (CAK) seated 20 m away recorded all vocalizations and narrations of all physical movements aided by flags hung at 1 m and 5 m using a Marantz PMD 660 solid state digital recorder attached to a Sennheiser ME62 omnidirectional microphone and a Mineroff parabolic reflector. The sex of responding birds was easy to determine using color bands and sex-specific vocalizations. If the bird was not seen or heard during the entire 15 minute trial, we dropped them from all analyses. For birds that had several different mates over the season, we only considered the first test of each trial type in further analysis. We tested all individuals between sunrise and 12:30 EST within the first four days of the female's egg laying period.

For each trial type, each bird heard a randomly selected playback stimulus from the set being used that year (2012: 6 heterospecific, 6 female, 6 male stimuli; 2013: 6 heterospecific, 15 female, 14 male stimuli; 2014: 6 heterospecific, 9 female, 8 male stimuli). Every playback stimulus contained 6 high quality song recordings from a single individual, each separated by 5 second of silence, filtered at 1 kHz, standardized to a peak amplitude of 1.4 kU using Raven Pro v1.4 (Bioacoustics Research Program 2011), and played back at a peak amplitude of 70-75 dB measured at 1 m using an Extech digital sound level meter (model 407732).

Birds were never played their own song, songs of an immediate neighbor, or playback stimuli they heard in a previous year. We assigned trial order randomly and conducted only one trial per day. Females did not change their responses between the first and second conspecific trial they heard (after controlling for the effect of trial type). Furthermore, female responses were not significantly different between females that heard female trials first and females that heard male trials first (gamma generalized linear model; $N = 123$ female trials, 113 male trials, order: $\chi^2 = 2.244$, $P = 0.13$; trial type by order interaction: $\chi^2 = 1.51$, $P = 0.22$). Males were similarly unaffected by trial order (gamma generalized linear model; $N = 112$ male trials, 112 female trials, order: $\chi^2 = 0.098$, $P = 0.95$; trial type by order interaction: $\chi^2 = 0.953$, $P = 0.62$). For additional details, see Krieg & Getty (2016), Chapter 2, “*Playback Stimuli*”, “*Playback Experiment*”, and “*Aggression Score*”, and Chapter 3, “*Aggression Trials*”.

Aggression Score

We assigned individuals of each sex an aggression score based on four behaviors that are commonly used as proxies for aggressiveness in playback studies: attacks, dives, latency to approach speaker within 5 m, and the difference in time spent within 1 m of the tripod and speaker during the playback period compared to the pre-playback period. We defined dives as

swooping rapidly within several centimeters of the model or the speaker. Attacks required actual physical contact. We calculated scores using the formula below. Physical aggression (dives and attacks) were weighted more heavily in the calculation of aggression scores as these are clearly a more aggressive behavior than merely approaching the speaker. For more details on aggression scores see Chapter 2, “*Aggression Score*”. All future references to aggression or aggressiveness refer to the responses to the same-sex playback trial.

$$\text{Aggression score} = 3 * (\# \text{ of attacks}) + 2 * (\# \text{ of dives}) + 1 * ((300 \text{ sec} - \text{sec to approach within 5m}) / 300 \text{ sec}) + 1 * ((\text{sec within 1m during playback} - \text{sec within 1m during pre-play}) / 300 \text{ sec})$$

Although we have previously documented that females adjust their level of aggressiveness based on the playback stimulus used and box treatment (see Chapter 2 and 3 for details), these factors do not describe all the variation in female aggression. A generalized linear model with a gamma distribution examining the effect the box treatment and stimulus on all female aggression scores from 2012-2014 had a likelihood ratio pseudo- R^2 value of 0.33, indicating a large portion of the variation is unexplained by these two factors. Gamma generalized linear models are suitable for continuous data with right skewed errors (Bolker 2008).

Eviction Experiment

To determine whether female aggression is an asset during periods of high competition, we performed a population scale manipulation of competition in 2013. We paired territories based on the date of the first egg was laid and randomly assigned one territory to the eviction treatment ($N = 17$ pairs). After testing aggression as described above (“*Aggression Trials*”), we

placed a cork in the entrance of all boxes on the eviction treatment territories. This prevented the adults from returning to the box. Nestboxes on eviction treatment territories then remained closed for the entire season. We transferred the eggs left in the eviction treatment box (1-4 eggs) to the un-manipulated box to avoid destroying the eggs.

We began the same eviction experiment in 2014. However, the small population size that year left many empty territories for the evicted birds to occupy, making it unlikely these evictions significantly elevated competition on a population-wide scale. We stopped the 2014 experiment after just 7 evictions. We included these 2014 data in our analysis of individual takeover outcomes (see “*Successful Takeovers*” below) but did not include them in our population-wide analysis of ovicide rates (see “*House Wren Ovicide*” below).

Successful Takeovers

To determine whether aggression helps females win contests over territories, we compared the winner female aggression score to the loser female aggression score for known female takeovers in 2013 and 2014. We had insufficient records of female movement in 2012 to examine takeovers in this year. We determined that a female takeover had occurred if the following three criteria were met. First, we observed a female with new color bands on the territory. Second, we had evidence that the old female had occupied the territory during our previous visit to the box based on either direct observation of the old female’s color bands or the presence of a new egg in the undisturbed nest. This reduces the likelihood that we included females who discovered empty territories long after the original female abandoned the nest. Third, we found the eggs missing from the nest, but the classic signs of nest predation (e.g. box damage, egg shells) were not present. The takeover was considered complete when the new female initiated a clutch. Two cases of female takeover occurred prior to egg laying. In both

these instances, the original female was in the late stages of nest lining which occurs after courtship and just prior to egg laying (McCabe 1965). The presence of a new female was detected using criteria 1, 2, and the presence of new sticks and lining in the nestbox (Alworth & Scheiber 2000). It is possible additional takeovers occurred that did not meet these criteria. Furthermore, in several instances, we had not measured the aggression score of both opponents before the takeover occurred. Therefore, this data set examined only a subset of female takeovers. We used a paired Wilcoxon signed-rank test to compare the winner's female aggression score to the loser's female aggression score for 13 female takeovers.

House Wren Ovicide

As part of both successful and attempted takeovers, house wrens of both sexes will sometimes destroy other house wren eggs (Belles-Isles & Picman 1986). To determine whether female aggression helps females minimize this consequence of elevated competition, we examined the relationship between female aggression and the proportion of eggs lost to house wren ovicide in 2013. Predators and house wrens leave different evidence around the nest. Nests in our population are predated by a variety of small and medium-sized mammals (e.g. deer mice, flying squirrels, raccoons). We considered eggs lost to predation if (1) the box was visibly damaged, (2) the nest was clearly messy and disturbed, (3) shells were present but the rest of the eggs had been consumed, or (4) a nest predator was now living in the box. We considered eggs lost to ovicide if the eggs were (1) found cracked but otherwise unconsumed, (2) found with beak-sized punctures, (3) found unconsumed on the ground in front of the box, or (4) missing on the day a new wren moved on to the territory. If eggs were missing but did not meet any of these criteria, we considered these "unknown source of destruction" and did not include them in this analysis.

To determine whether female aggression score affects the proportion of eggs lost to house wren ovicide, we used a generalized linear model (GLM) with a binomial distribution with male aggression and day in the season included as covariates. Binomial GLM's are appropriate for proportional data that can be considered a series of "successes" and "failures" (Bolker 2008). One male in the data set attacked the playback setup before the playback even started. This male had a very high aggression score and was a significant outlier in the model as determined by Cook's distance in the residuals vs. leverage plot. We therefore removed this point from this analysis. However, including this point did not change the results regarding female aggression or day in the season, although it did make the effect of male aggression highly significant. We also ran a simpler binomial generalized linear model looking at just the effects of (1) whether females engaged in physical aggression (i.e. dives and attacks) during female playback and (2) day in the season. In both models, VIF values were less than 2, indicating no problems with multicollinearity. Because binomial generalized linear models do not produce the R^2 values used in traditional linear models, we instead calculated goodness-of-fit with pseudo- R^2 values based on a likelihood ratio test (Magee 1990) using the "r.squaredLR" command in the package "MuMIn" (Barton 2015). We performed all statistical tests in R version 3.0.2 (R Core Team 2013; package 'car': Fox & Weisberg 2011; package 'multcomp': Hothorn et al. 2008).

Ethical Note

This research was approved by the Michigan State University's Institutional Animal Care and Use (ACUC) panel. Field work was conducted under appropriate federal and state permits (MSU IACUC Animal Use Form numbers 04/11/076-00, 03/14-062-00; U. S. Fish and Wildlife Service Federal Banding Permit number 23587; Michigan Department of Natural Resources Scientific Collectors Permit number SC 1432, U. S. Fish and Wildlife Service Permit number

MB01550B-1). No females abandoned territories following our tests. No birds suffered adverse effects during capture and measurement.

RESULTS

Female and male house wrens were most aggressive towards simulations of same-sex intruders. Females had higher aggression scores during female playback trials than male or heterospecific trials (gamma generalized linear model: trial type: $\chi^2 = 48.375$, $P < 0.0001$). Similarly, males had the highest aggression scores during male trials (gamma generalized linear model: trial type: $\chi^2 = 140.88$, $P < 0.0001$). For more details on female and male aggression scores, see Appendix A.

Territory Takeovers

Females that were aggressive toward simulated female intruders were significantly more likely to win subsequent territory takeovers ($N = 13$ takeovers, $V = 78$, $P = 0.025$; Figure 4.1). Winner aggression scores were lower than loser scores in only three instances. In two of these instances, one bird won possession of territory and initiated a clutch only to be usurped by the original resident 5 to 8 days later. In all cases where more aggressive birds lost, the absolute difference in aggression was less than one score point. This is a relatively minor difference considering female aggression scores in 2013 ranged from -1.02 to 8.25. No losing females ever escalated to dives or attacks during the simulated female intrusion. Perhaps unsurprisingly, the majority of these takeovers occurred during 2013 when competition was experimental elevated (9 out of 13).

It is possible that females can only win takeovers on territories where the resident males are not aggressive. However, our data suggest this is not the case. Male aggression scores on

territories where female takeovers occurred were approximately the same as male aggression scores for males on territories where female takeovers did not occur (female takeover male score: 2.497 ± 1.202 (SE), no female takeover male score: 2.628 ± 0.321 (SE); Mann-Whitney test: $W = 259$, $P = 0.9135$). In all instances, the original male remained on the territory after the female takeover. Instead, the relative aggression scores of the intruding female and the resident female appear to be important determinants of which female ultimately possessed the territory.

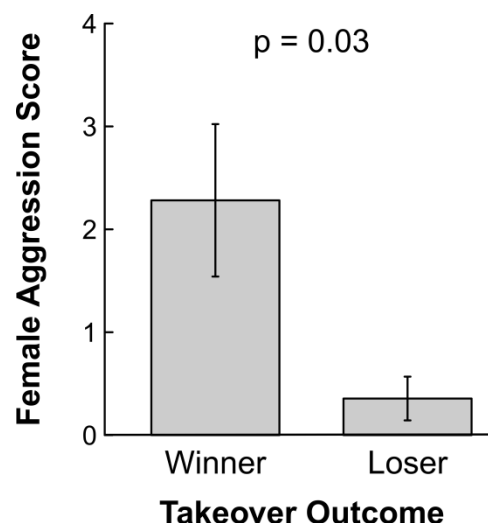


Figure 4.1: Females that won territory takeovers had significantly higher aggression scores than females that lost (Wilcoxon signed-rank test, $N = 13$, $P = 0.03$). Error bars represent standard errors of the mean.

House Wren Ovicide

The population-wide manipulation of competition dramatically increased the number of eggs lost to house wren ovicide in 2013 compared to 2012 (Figure 4.2). Fewer eggs hatched in 2013 (47.7% hatched) than 2012 (70.4% hatched). In both years, predation was the main cause of egg loss. However, predation was roughly the same between both years (2012: 18.6% eggs lost to predation, 2013: 20.6% eggs lost to predation). In comparison, house wren ovicide losses increased more than three-fold (2012: 4.4% eggs lost to ovicide, 2013: 15.4% eggs lost to

ovicide; for additional details see Appendix B). Most of the ovidice losses occurred around the time evicted birds were re-entering the floater population (Figure 4.2).

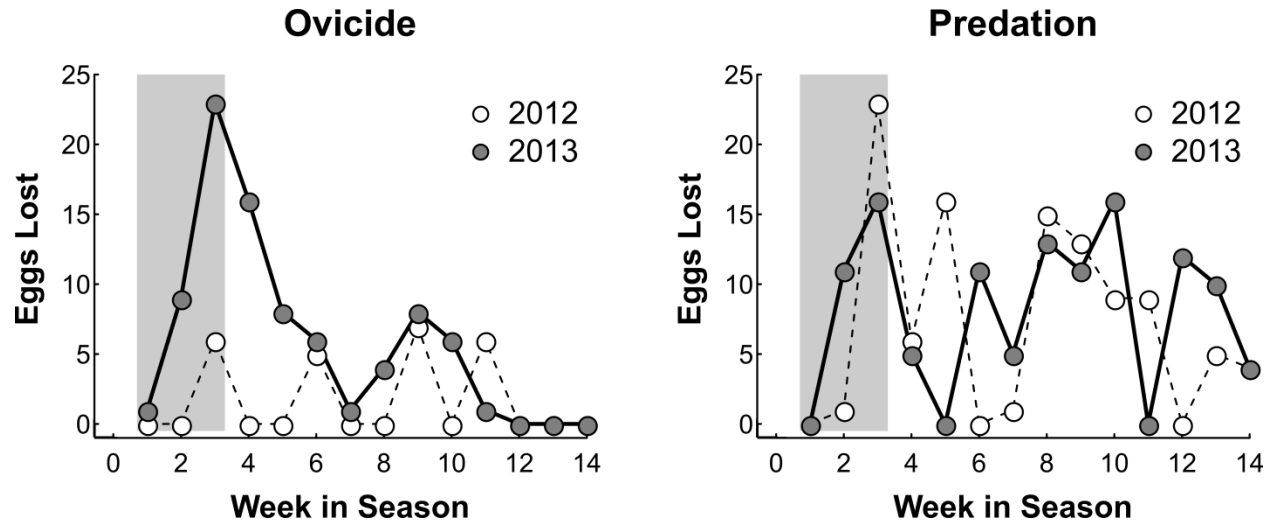


Figure 4.2: The number of eggs lost to house wren ovidice and predation over the course of the season. Wrens were experimentally evicted during the weeks shaded in gray in 2013 only. Ovidice losses dramatically increased following the eviction period in 2013. Week one was defined as the first week an egg was laid at the study site (May 4th, 2012; May 8th, 2013).

Females with higher aggression scores lost significantly fewer eggs to house wren ovidice than less aggressive females after controlling for male aggression score and day in the season (Figure 4.3a, Table 4.1; female aggression score: $N = 64$ pairs, $\chi^2 = 4.704$, $P = 0.03$, model likelihood ratio pseudo- $R^2 = 0.30$). Furthermore, all the females that suffered complete clutch losses due to a successful territory takeover by a floating conspecific did not engage in physical aggression (i.e. dives and attacks) during the female aggression trials. While female aggression significantly predicted the proportion of eggs lost to ovidice, male aggression had no effect after accounting for the significant effects of female aggression and day in the season (Table 4.1). Day in the season also had a significant effect on ovidice losses due the timing of evictions at the beginning of the season (Figure 4.2).

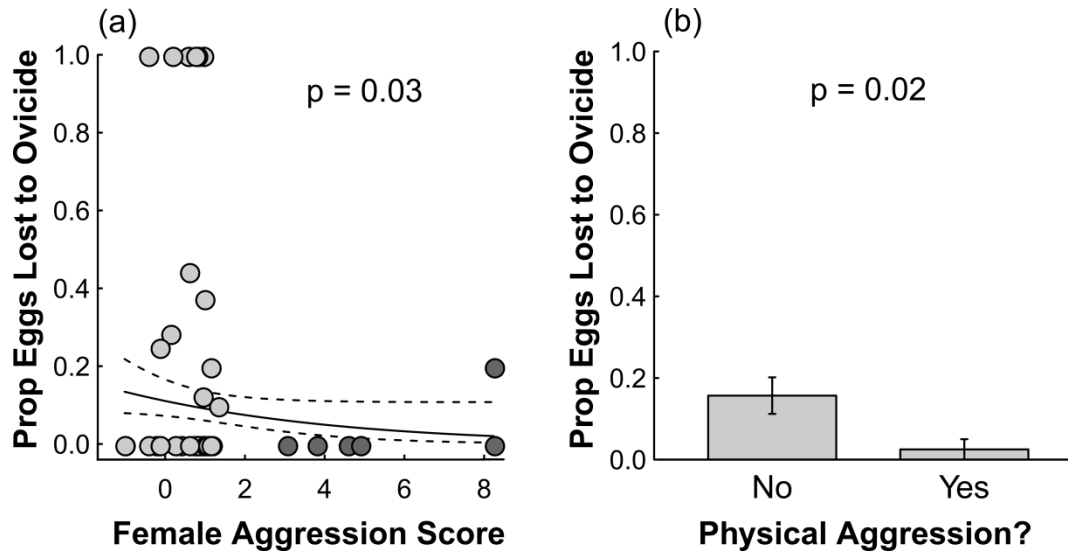


Figure 4.3: The relationship between the proportion of eggs lost to house wren ovicide in 2013 ($N = 64$) and (a) the resident female aggression score, or (b) the occurrence of physical aggression (whether females dove at or attacked the female playback). Lines in (a) represent predicted values $\pm$ 95% CI from a binomial generalized linear model. Dark points in (a) indicate birds that engaged in physical aggression. Error bars represent standard errors of the mean.

Table 4.1: Results from binomial generalized linear models showing effects of day in the season, and aggression scores of females and males on the proportion of eggs lost to house wren ovicide. Bolded values indicate $P < 0.05$.

Variable	Estimate $\pm$ SE	χ^2	P
Resident female aggression score	-0.217 $\pm$ 0.113	4.704	0.030
Resident male aggression score	0.052 $\pm$ 0.057	0.789	0.374
Day in season	-0.037 $\pm$ 0.011	17.553	< 0.001

When we restrict our analysis of aggression to dives and attacks at the simulated female intruder, females who dove and attacked more still lost fewer eggs to ovicide than those who were not physically aggressive (Figure 4.3b; $N = 64$ females; dives and attacks; estimate $\pm$ SE = -1.453 $\pm$ 0.748, $\chi^2 = 5.531$, $P = 0.019$; day in season: estimate $\pm$ SE = -0.038 $\pm$ 0.01, $\chi^2 = 18.436$, $P < 0.001$, model likelihood ratio pseudo- $R^2 = 0.31$). This indicates that our results do not merely represent an artifact of the aggression score calculation. Only one female that engaged in

physical aggression with the simulated female intruder lost any eggs to house wren ovicide. However, this female only suffered partial, rather than total, clutch loss.

DISCUSSION

Female house wrens that were aggressive towards female intruders were more successful than others when competition was experimentally enhanced. The female with the highest aggression score was most likely to win a contest over a nesting cavity. Aggressive females also coped better with the consequences of elevated competition. Evicting pairs and limiting the number of nest cavities in the population in 2013 resulted in dramatically higher rates of house wren ovicide in that year than 2012. Females that were more aggressive towards simulated female intruders lost fewer of their eggs to house wren ovicide than less aggressive females. Somewhat surprisingly, however, aggression by females, but not by males, predicted future ovicide losses. These results suggest that intrasexual aggression among females may be under selection through competition for nesting cavities in house wrens.

Although we experimentally forced some birds to seek new cavities, this is likely a challenge faced by females under natural conditions. Females arrive at the study site asynchronously at the beginning of the season. Females in this population are also often double-brooded. Females often switch territories between broods (58% of double-brooded females in 2012, 56% in 2014), although the relative numbers forced to do so by female intruders and those that freely choose to leave are unknown. Furthermore, premature clutch losses due to predation make the timing of the second brood asynchronous within the population, meaning that resident females may be faced with potential intruders throughout the majority of the season. How easy it is to find a new cavity likely depends on the population size that year, which at our study site

has varied between 33 and 71 females in the last seven years, depending on the year. Although intrasexual aggression may not benefit females in years when competition is relaxed, our data suggest it can have a substantial benefit when competition is intense.

Female Aggression across Contexts

Territorial aggression and maternal aggression (aggression in defense of eggs or offspring) are typically treated as separate phenomenon (Cain et al. 2011, Huntingford 1976). However, data from other avian species as well as work on behavioral syndromes suggest that aggression in one context might sometimes be part of a general tendency to be aggressive across contexts (Bell 2007, Cain et al. 2011, Duckworth 2006, Sih et al. 2004a, Sih et al. 2004b). Whether our assay measures territorial behavior or maternal aggression is somewhat unclear, given its timing at the beginning of egg laying. At this point in time, female intruders could destroy eggs in the nest, usurp the nesting cavity, or threaten future paternal investment by settling as a secondary female. Regardless of which motivation is primarily driving female responses to simulated female intruders in this study, female aggressiveness in this assay correlated with the ability both to win territories and to protect offspring. Furthermore, we found that our single measure of aggressiveness early in the nesting cycle correlated with outcomes throughout the breeding season, suggesting we captured a general characteristic of the female phenotype rather than a fleeting response to experimental playback. Intrasexual aggressiveness may be just one component of a generally aggressive phenotype in female house wrens. Multiple tests of female aggression across the nesting cycle in response to a wider variety of stimuli would be necessary to test this hypothesis.

Female Aggression and Sexual Selection

Recent focus on intrasexual competition in females has advanced several important concepts in the field of sexual selection. The finding that female aggression can affect a female's success at gaining and keeping a territory may appear unsurprising, however, patterns like these have important implications for the seemingly unrelated topic of mate choice. Classic models of female mate choice assumed that females freely chose among male in order to maximize their own fitness (Leonard 1990, Orians 1969, Verner & Wilson 1966, Weatherhead & Robertson 1979). In such models, females are assumed only to influence the mate choices of other females in so far as they might deplete the resources that territorial males can offer in the future. If females compete aggressively and can interfere directly with the choices of other females, however, the predictions from these models change dramatically. Models explicitly incorporating female competitive strength do a much better job at explaining female choices in multiple species that do not match the predictions from older, free choice models (Dale et al. 1992, Frame 2012, Grønstøl et al. 2003, Slagsvold & Lifjeld 1994). Emphasis on competition among females has led to the appreciation that it is possible for members of one sex to be both choosy and competitive simultaneously (Almada et al. 1995, Bro-Jørgensen 2011, Owens & Thompson 1994, Sæther et al. 2001).

House wrens are one of a growing list of species in which female compete aggressively with other females for access to critical breeding resources. Cavities are often critical, limited resources for populations of cavity nesting birds (Newton 1994). Females in other cavity nesting species compete aggressively with other females for access to nest cavities (Heinsohn et al. 2005, Heinsohn 2008, Rosvall 2008). A wide variety of other taxa compete for nest or oviposition sites as well (arachnids: Elias et al. 2010; fish: Borg et al. 2002; insects: Shelly 1999; non-cavity

nesting birds: Renison et al. 2003, Robinson 1985; reptiles: Rubenstein & Wikelski 2005).

Likewise, when female fitness is limited by other resources, females compete with other females for access to those as well. Females may compete over paternal care (Arcese 1989, Ekstrom et al. 2007, Liker and Székely 1997, Sandell 1998, Wong et al. 2008,), social rank (Clutton-Brock et al. 2006, Clutton-Brock & Huchard 2013, Young and Bennett 2013), food resources to invest in reproduction (Eggert et al. 2008, Gwynne & Simmons 1990, Pusey & Schroepfer-Walker 2013, Robinson & Kruuk 2007, Watson & Simmons 2010), and even access to sperm limited males (Bro-Jørgensen 2002, Papadopoulos et al. 2009). Darwin devotes hundreds of pages of his *“The descent of man and selection in relation to sex”* to describing the “Law of Battle” that fuels male competition for females (Darwin 1871). Renewed focus on female behavior reminds us that Darwin’s “Law of Battle” applies in a rich diversity of contexts.

Here we show that more aggressive female house wrens are both directly more successful when competing for limited nest cavities and more successful at dealing with the consequences of intense competition. Much of the recent literature on intrasexual competition among females has focused on determining whether female competitive traits are under sexual selection or social selection (Carranza 2009, Carranza 2010, Clutton-Brock 2007, Clutton-Brock 2009, Forsgren 2011, LeBas 2006, Robinson 2011, Rosvall 2011a, Rosvall 2011b, Shuker 2010, Stockley & Bro-Jørgensen 2011, West-Eberhard 1983, While 2011). Some have argued that intrasexual competition for resources that allow females to breed at all is analogous to male competition for courtship display sites or territories (Clutton-Brock 2007, Rosvall 2011a). The later is typically considered under the umbrella of sexual selection. However, female competition to protect offspring does not fit neatly into the sexual selection narrative. Because females often compete for resources that may provide multiple types of benefits (e. g. access to breeding opportunities,

improved offspring growth, etc.), and because the same female trait may benefit females at multiple reproductive stages, it may be particularly difficult to find the boundaries between sexual and social selection in regard to females (Clutton-Brock & Huchard 2013, Lyon & Montgomerie 2012, Tobias et al. 2012). Perhaps it would instead be more fruitful to recognize that a single trait, whether in males or females, may influence various components of fitness (Cain & Rosvall 2014, Clutton-Brock 2010, Clutton-Brock & Huchard 2013). Because they have received less study, competitive female phenotypes may have benefits for females that have previously been underappreciated (Clutton-Brock 2007, Rubenstein 2012). However, the specific costs and benefits to these phenotypes may very well differ between males and females of the same species (Cain & Rosvall 2014, Carranza 2009, Clutton-Brock 2010, Clutton-Brock & Huchard 2013, Robinson 2011, While 2011). Further study of female behavior is critical to understanding the evolution of social behavior as a whole.

CHAPTER 4 ACKNOWLEDGEMENTS

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APPENDICES

APPENDIX A

Additional details on aggression trials with house wrens

Females

Female responded significantly more aggressively during simulations of female intruders than simulations of male intruders or heterospecifics (Figure 4.4a; gamma generalized linear model: trial type: $\chi^2 = 48.375$, $P < 0.0001$). This analysis included all females that were tested between 2012 and 2014. Any female that was neither seen nor heard during the entire 15 minute trial was excluded from this data set (final heterospecific trials = 112, female trials = 123, male trials = 113). Whether females had heard another house wren trial on a previous day did not affect their aggression scores in subsequent trials (gamma generalized linear model; order: $\chi^2 = 2.287$, $P = 0.13$; trial type by order interaction: $\chi^2 = 1.64$, $P = 0.2$).

The differences between trial types were still apparent if we broke aggression scores into separate behaviors (Figure 4.4b-d). We used a gamma generalized linear model for the latency to approach within 5m. We were unable to find a suitable parametric model for dives and attacks and the difference in time spent within 1m in the playback period compared to the pre-playback period. For these variables, we used Mann-Whitney tests with Holm's p-value corrections to correct for multiple comparisons (Holm 1979). Individual aggression score components were generally weakly correlated with one another (Table 4.2), although females who approached faster generally spent more time within 1 m (all trials) and engaged in more physical aggression (female trials only).

Table 4.2: Pearson's correlation coefficients (r) for individual components of the female aggression scores during each playback type (** $P < 0.001$, * $P < 0.05$). Bolded values indicate $P < 0.05$.

Variable pair	Female playback	Male playback	Heterospecific playback
Latency to approach, time within 1m	-0.324**	-0.185*	-0.191*
Latency to approach, dives/attacks	-0.201*	-0.121	-
Time within 1m, dives/attacks	0.175	-0.054	-

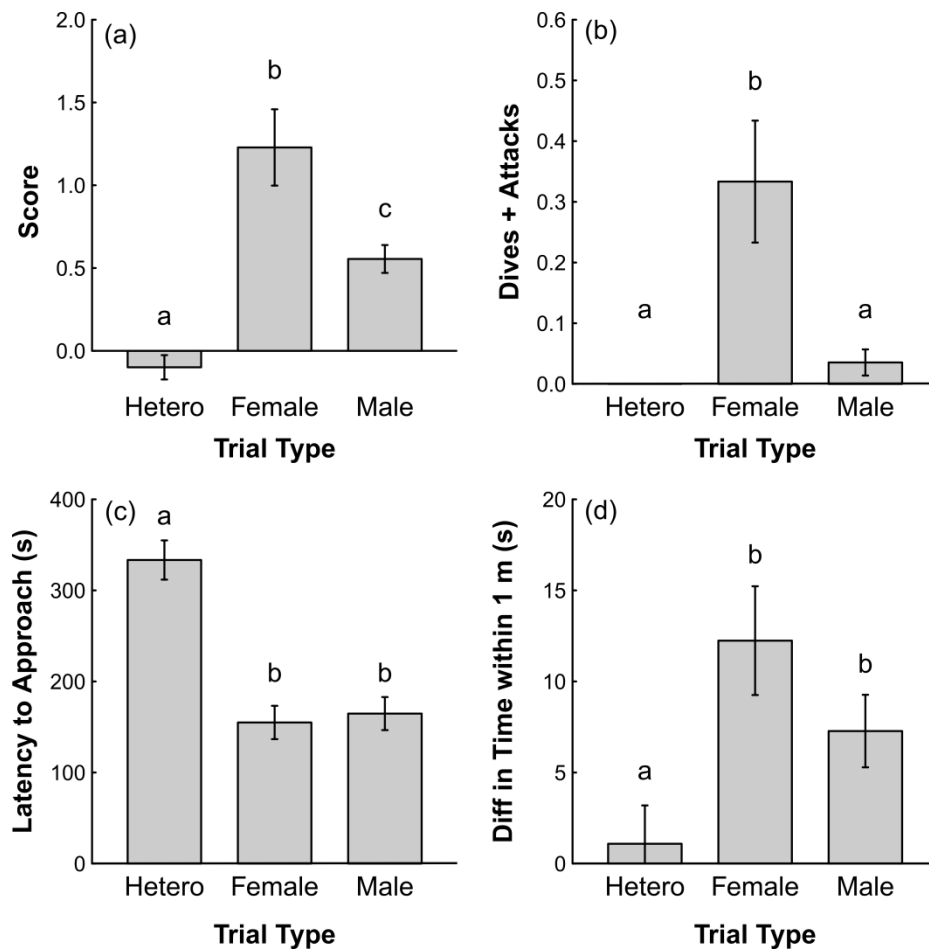


Figure 4.4: Mean $\pm$ SE female aggression scores (a), dives and attacks (b), latency to approach within 5 m (c), and the difference in time spent within 1 m during the playback period compared to the pre-playback period (d) based on trial type. Letters represent Tukey's posthoc contrasts from a gamma generalized linear model in (a) and (c). Letters represent results from Mann-Whitney tests with Holm's corrected p-values in (b) and (d). The difference between male and female trials were nearly significant in panel (d) (corrected $P = 0.05987$).

Although a female's score during the simulation of a female intruder and the simulation of a male intruder were correlated, the correlation was weak (Pearson's correlation coefficient = 0.2, $P = 0.04$; Figure 4.5a). This suggests female trials do not solely measure a female's general tendency to respond to intruding conspecifics. Some females were highly aggressive towards female simulations, but not males, while others were only aggressive towards male simulations (Figure 4.5a). A female's score during the female trial was not correlated with the female's score during the heterospecific trial (Pearson's correlation coefficient = 0.06, $P = 0.51$; Figure 4.5b). This suggests that the female's score during the female trial does not capture a female's tendency to move around near the nestbox in the absence of same-sex competitors.

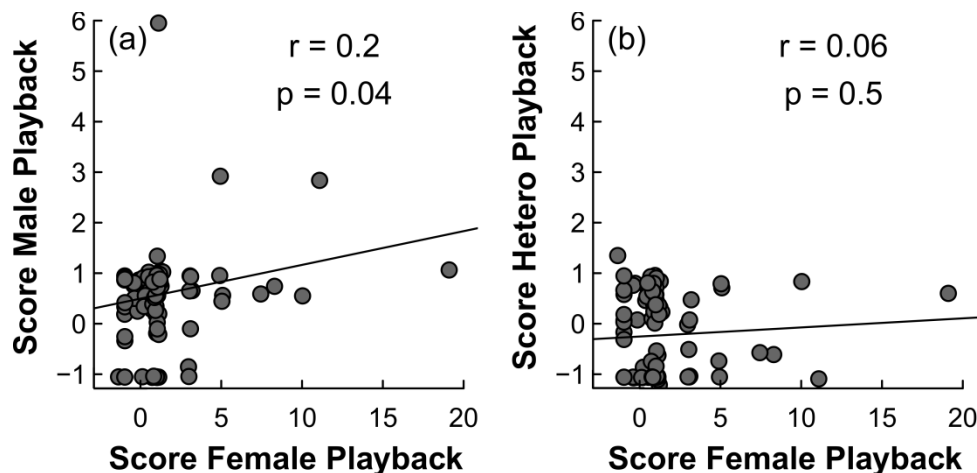


Figure 4.5: The correlation between a female's aggression score during female playback trials and her score during male playback trial (a) and heterospecific playback trial (b) with Pearson's correlation coefficients.

Males

Like females, male house wrens responded significantly more aggressively during simulations of same-sex intruders than opposite-sex or heterospecific intruders (Figure 4.6a; gamma generalized linear model; trial type: $\chi^2 = 140.88$, $P < 0.0001$). As with the female analysis, we dropped all males neither seen nor heard during the trial (final heterospecific trials = 108, female trials = 112, male trials = 111). Overall, male aggression towards same-sex intruders was more intense than female aggression in the same context. This is true when examining overall aggression scores as well as individual aggression score components (Mann-Whitney tests; score: $W = 3952$, $P < 0.0001$; dives and attacks: $W = 4894$, $P < 0.0001$; latency to approach: $W = 9725.5$, $P < 0.0001$; time within 1m: $W = 5602$, $P = 0.014$). Exposure to previous house wren trials did not affect male aggression scores in subsequent trials (gamma generalized linear model; order: $\chi^2 = 0.101$, $P = 0.95$; trial type by order interaction: $\chi^2 = 0.964$, $P = 0.62$).

Unlike female house wrens which respond to the opposite-sex intruders with intermediate levels of aggressiveness, male house wrens appear to only react aggressively towards same-sex intruders (Figure 4.6). Male responses to heterospecific and female intruders did not differ when it came to male aggression scores, the number of dives and attacks, and the time spent within 1 m. In fact, only one male ever dove at a simulated female intruder. Males did approach simulated female intruders significantly faster than heterospecific intruders but significantly slower than male intruders (Figure 4.6c). Males may view these simulated female intruders as potential mates. We used the same analyses as described in the female section above.

Male aggression scores during simulations of male intruders do not appear to capture a general reaction to intruding conspecifics nor a general tendency to move around the nest. Male

aggression scores during male trials were not correlated with scores during female trials or heterospecific trials (male and female scores: Pearson's correlation coefficient = 0.07, $P = 0.45$, Figure 4.7a; male and heterospecific scores: Pearson's correlation coefficient = 0.18, $P = 0.07$; Figure 4.7b).

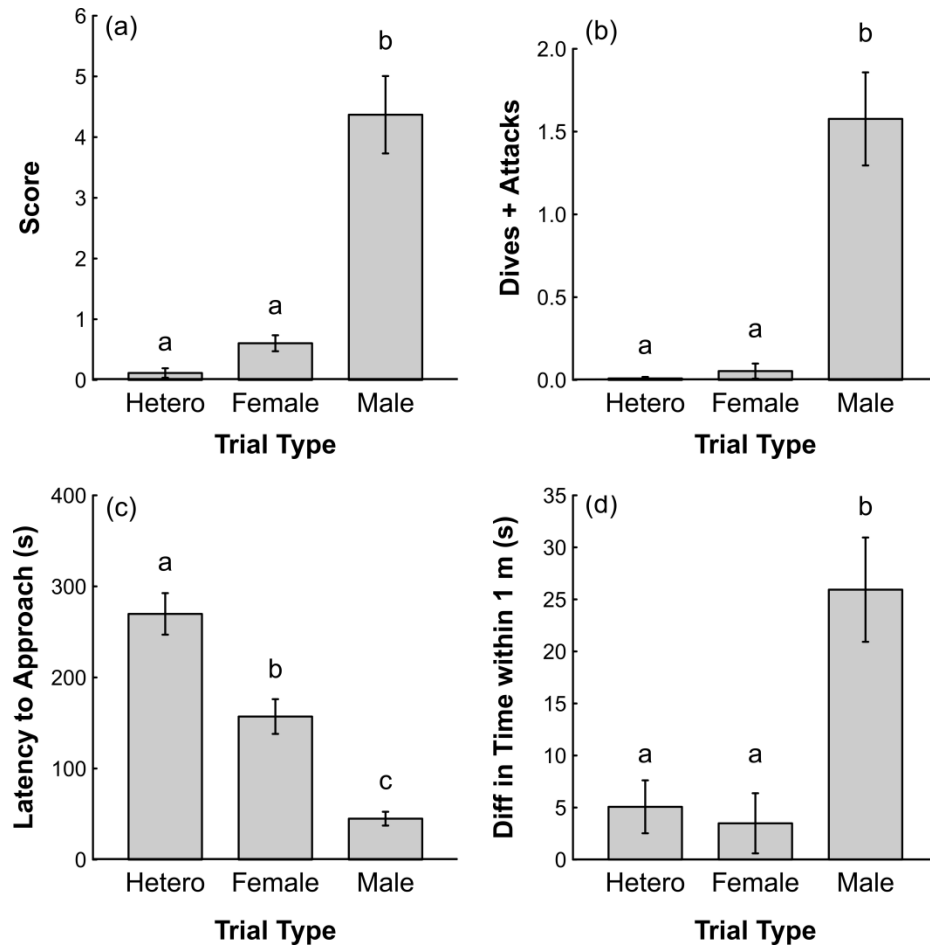


Figure 4.6: Mean $\pm$ SE male aggression scores (a), dives and attacks (b), latency to approach within 5 m (c), and the difference in time spent within 1 m during the playback period compared to the pre-playback period (d) based on trial type. Letters represent Tukey's posthoc contrasts from a gamma generalized linear model in (a) and (c). Letters represent results from Mann Whitney tests with Holm's corrected p-values in (b) and (d).

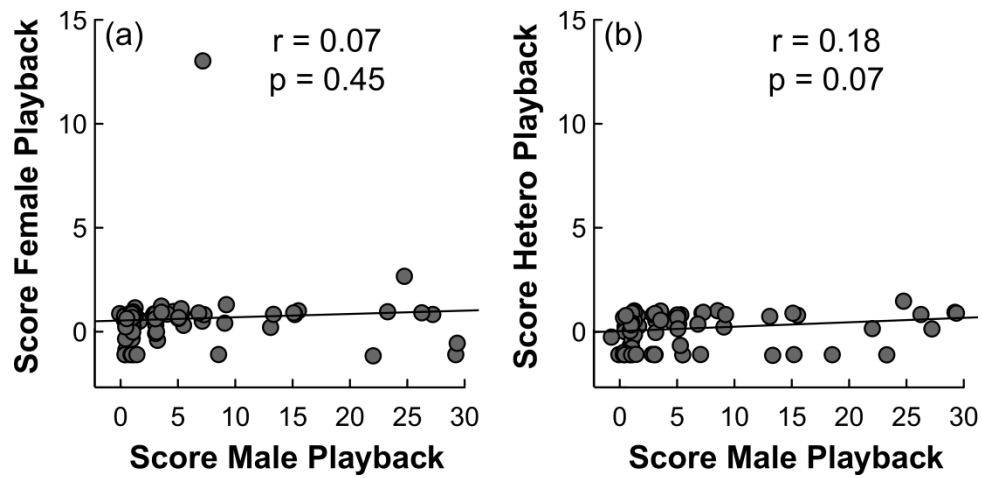


Figure 4.7: The correlation between a male's aggression score during male playback trials and his score during female playback trial (a) and heterospecific playback trial (b) with Pearson's correlation coefficients.

APPENDIX B

House wren egg losses in 2012 and 2013

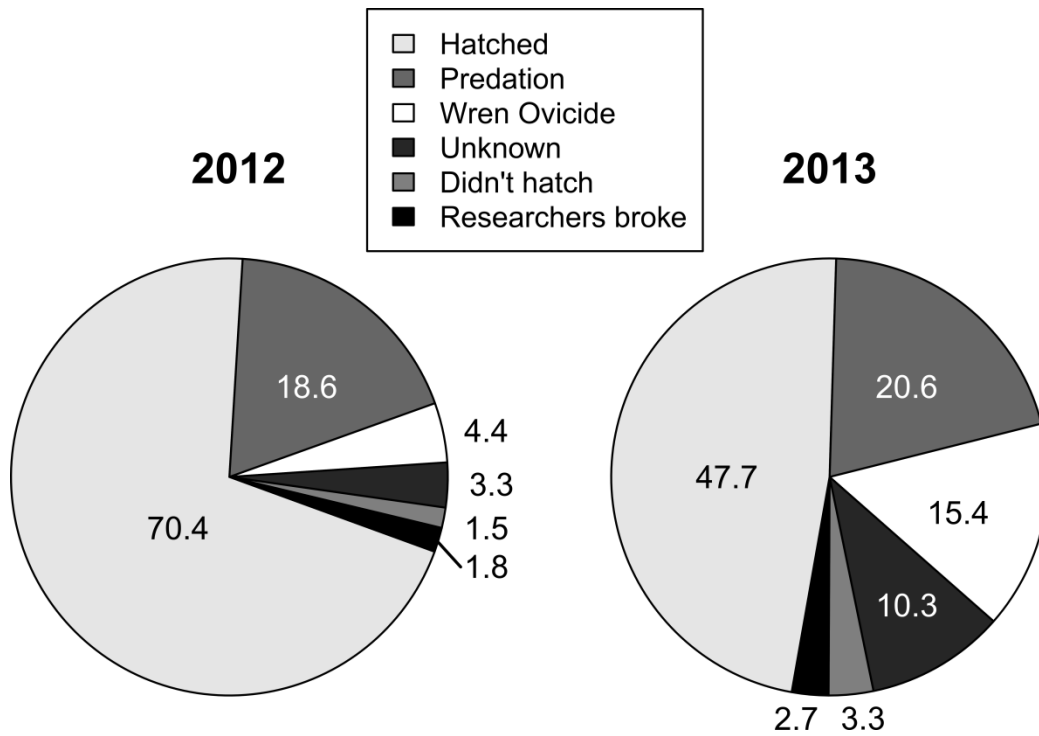


Figure 4.8: Fate of house wren eggs laid in 2012 and 2013 (competition experimentally elevated). Numbers inside slices indicate the percentage of eggs with that fate. House wrens laid approximately the same number of eggs in both years (2012: 550 eggs, 2013: 553 eggs). Eggs lost for unknown causes were likely due to either predation or wren ovide, but the evidence was inconclusive.

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CHAPTER FIVE

Fitness consequences of intrasexual aggression in female house wrens

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ABSTRACT

Males frequently compete aggressively with other males for access to mates, sometimes at a cost to parental behavior and self-maintenance. Theory predicts that a trade-off between aggressive competition and future reproductive investment should make aggressive competition among females less common and less intense than aggression among males. However, the reproductive consequences of female aggression across the reproductive cycle are unknown in many empirical systems. In house wrens (*Troglodytes aedon*), females respond aggressively to simulated female conspecifics. We have previously found that females who are more aggressive towards simulated female intruders are more successful at competing for limited nesting cavities when competition is experimentally elevated. Here we investigated additional fitness consequences to intrasexual aggression across the female reproductive cycle. Female aggression had no detectable relationship with male mate quality or maternal investment in egg production. However, aggression had benefits once nestlings hatched. Offspring of aggressive females were significantly heavier throughout development, even when demands on parents were increased by experimentally enhancing brood size. Nestlings from aggressive females were also significantly more likely to survive until fledging. Together with the effects documented in Chapter 4, eggs laid by aggressive females were significantly more likely to result in fledged offspring. While aggressive females appear more successful within a given season, our data suggest that they may

be less likely to return from migration the next year. The mechanisms underlying the benefits to offspring are unknown, although they could come from increased predator defense, increased foraging efficiency, maternally deposited androgens, or other by-products of the physiological mechanisms regulating intrasexual aggression. If intrasexual aggressiveness is heritable and the within season benefits outweigh the potential longer-term costs, female aggressiveness could be under selection in house wrens.

INTRODUCTION

Aggressive competition between members of the same sex is generally considered a male endeavor. Because male fitness is often strongly influenced by access to mates, males in many species have evolved aggressive behavior as well as ornaments and armaments used in competition (Andersson 1994, Bateman 1948, Darwin 1859, Darwin 1871, Shuster & Wade 2003, Trivers 1972). Females in most conventional sex-role species do not face a shortage of mates (Shuster & Wade 2003). Therefore, female-on-female aggressive competition for mates is typically absent or very mild.

Aggressiveness is just one aspect of a male phenotype that affects many other male characteristics. In many species, male aggression is mediated by testosterone (or other androgens) (Hau 2007). Circulating testosterone acts on a wide variety of tissues in the periphery and the central nervous system (Staub & De Beer 1997, Wingfield et al. 2001). Testosterone in males enhances sexual behavior, boosts metabolic rate, enhances muscle growth but also, in some species, reduces parental behavior and suppresses immune function (Casto et al. 2001, Clark & Galef 1999, Folstad & Karter 1992, Greives et al. 2006, Hau 2007, Ketterson et al. 1992, Lynn 2008, Marler & Moore 1988, McGlothlin et al. 2007, Nunes et al. 2001, Prall &

Muehlenbein 2014, Wingfield et al. 2001). Little is known about the hormonal-mediation of female aggression, although evidence suggests that circulating androgens could play a role in some species (Albert et al. 1992, Beehner et al. 2005, Cain & Ketterson 2012, Geslin et al. 2004, Glickman et al. 1992, Langmore et al. 2002, Rosvall 2013b, Sandell 2007, Staub & De Beer 1997, Zysling et al. 2006; but see Elekonich & Wingfield 2000, Goymann et al. 2008, Rosvall et al. 2012, While et al. 2010).

The consequences of elevated androgens in males have been invoked to explain why aggression among females is typically less common and less intense than aggression among males (Clutton-Brock & Huchard 2013, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013). Because variation in female reproductive success is primarily driven by limits to fecundity, rather than access to mates, female-female competition lacks the sexual benefits of male competition (Bateman 1948, Shuster & Wade 2003, Trivers 1972). Furthermore, females are predicted to face a fecundity cost if they invest limited time and energy in competition rather than reproduction (Fitzpatrick et al. 1995, Trivers 1972). Females may also face additional physiological costs from the androgens predicted to underlie female aggression. Experimental elevation of testosterone in female birds reduces fecundity (Rutkowska et al. 2005, Gerlach & Ketterson 2013), diminishes mating preferences (McGlothlin et al. 2004), slows the rate of feather growth and molting (De Ridder et al. 2002, Clotfelter et al. 2004), alters incubation behavior (Rosvall 2013b, O'Neal et al. 2008), and suppresses immune function (Zysling et al. 2006) (reviewed in Ketterson et al. 2005). Naturally occurring androgens also reduce adult and offspring survival in some mammal species (Altmann et al. 2004, Frank et al. 1995, Packer et al. 1995). With fewer sexual benefits than male aggression in the face of these costs, intense female aggression is predicted to be rare.

Nevertheless, female-female aggression is actually fairly common in a wide variety of taxa. Although much male-male aggression involves competition for mates, females may fight for a variety of reasons. Females may compete over access to particular males offering direct or indirect benefits, or, more commonly, females may compete over access to critical breeding resources (Berglund et al. 1993, Clutton-Brock 2007, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, Rosvall 2011b, Rubenstein 2012, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012, Watson & Simmons 2010). The nature of female competition may explain how females have avoided some of the costs predicted to constrain the evolution of female competitive traits. If females compete for access to resources that enhance female fecundity, then investment in competitive traits can enhance, rather than reduce, maternal performance (Cain & Ketterson 2013a, Cain & Rosvall 2014, Watson & Simmons 2010). Aggressive competition and maternal investment are not necessarily in conflict (Stiver & Alonzo 2009). To understand how female aggression evolves, it is necessary to understand the costs and benefits to female aggression both at the time of competition and throughout the female reproductive cycle.

In house wrens (*Troglodytes aedon*), females compete with other females for access to cavities and male care. House wrens are sexually monomorphic, facultatively polygynous cavity nesters with biparental care (Kendeigh 1941). Both males and females compete separately for access to nesting cavities (Johnson & Kermott 1990; Johnson & Searcy 1996, Chapter 4). Most females in our study population are double-brooded, and males provision nestlings during both broods (Johnson et al. 1993, Kendeigh 1941). We have previously determined that more aggressive females are more likely to win fights for limited nesting cavities and can better protect their eggs from intruding conspecifics when competition is experimentally elevated

(Chapter 4). However, female aggression could have important consequences during other points in the season.

Here we ask whether intrasexual aggression among female house wrens has fitness consequences beyond the initial competition for nesting cavities. Specifically, we ask whether more aggressive females gain access to higher quality mates and whether more aggressive females are ultimately more successful at fledging offspring. We then ask whether female aggression carries some of the costs that are associated with male-male aggression. Specifically, we ask whether female aggression affects maternal investment, female parental behavior, or nestling quality under both natural conditions and those in which demands on parents are experimentally elevated. Finally, we ask whether female aggression affects female survival.

METHODS

Study System and Study Site

This study was conducted with a house wren population nesting at the Lux Arbor Reserve at the Kellogg Biological Station in Barry County, MI (42° 29' N, 85° 28' W). This 529 hectare protected area contains forest, grassland, and fragmented agricultural fields. Each year since 1999, nestboxes have been provided in the same locations on suitable edge habitat (Dubois & Getty 2003). Each house wren territory contained a cluster of 3 nestboxes located within 5-10 m. Each box cluster was separated by at least 100 m from the next cluster, allowing the population to contain 59 house wren territories.

In 2012 and 2013, only one box was initially open on each territory. After male and female wrens settled on the territory, we randomly assigned half the territories to a three box treatment in an alternating fashion by removing the corks from the entrances of the 2nd and 3rd

boxes (for more details see Chapter 3). In 2014 all territories contained only one open box throughout the season. Box type did not affect any of the results discussed below.

The population contained 107 breeding adults in 2012 (56 females), 124 adults in 2013 (71 females), and 66 adults in 2014 (33 females). We trapped each bird on the nest or in a mist net, where we sometimes used male or female playback sounds as mist net lures. Mist nets were monitored continuously and no birds suffered adverse effects. If we captured birds prior to aggression testing, at least 3 days separated capture and testing. Each bird was given a USFWS band and three color bands to allow for individual identification. We measured mass to the nearest 0.1 g and tarsus to the nearest 0.01 mm. Condition was measured by taking the residuals from a regression of tarsus on to mass for each sex with nesting stage included as a covariate in the female model to account of systematic difference in female mass between incubation and non-incubation stages (for similar approach, see DeMory et al. 2010).

Territory Preference

Some territories were more likely than others to be occupied in a given year. We used the date on which the first egg was laid on a territory as an index of territory preference. The territory on which with the first egg of the season was laid was assigned a score of one, and each other territory was measured relative to that date (e.g. territory 1 first egg date: May 8th, score = 1; territory 2 first egg date: May 11th, score = 4). We gave territories that were never occupied a score of 100 to mark the end of the season. We then averaged the scores for each territory from 2008-2012 and 2014. We excluded 2013 since the experimental manipulations described in Chapter 4 very likely influenced the rate of territory settlement. Territories with lower scores were settled on average earlier than territories with higher scores. Territories with earlier settlement scores cumulatively produced more eggs, fledged more offspring, had a higher rate of

offspring survival, and were more likely to be targets for house wren takeovers in years that competition was not manipulated (see Appendix for analysis details). This suggests that territories with lower scores were higher quality resources. More aggressive females did not settle on more preferred territories after controlling for the significant effect of year (gamma generalized linear model: log transformed female aggression, $N = 109$, $\chi^2 = 0.001$, $P = 0.99$).

Aggression Trials

We assayed male and female aggressiveness towards simulated conspecific intruders using methods described in detail elsewhere (Krieg & Getty 2016, Chapter 2 “*Playback Stimuli*”, “*Playback Experiment*”, “*Aggression Score*”; Chapter 3 “*Aggression Trials*”; Chapter 4 “*Aggression Trials*”, “*Aggression Score*”). Briefly, a speaker and a wooden model house wren were placed within 1-2 m of the nestbox. Each trial started with 5 minutes of pre-playback silence, followed by 5 minutes of playback, and concluded with 5 minutes of post-playback silence. An observer seated quietly 20 m away recorded all vocalizations and narrated descriptions of any physical movements made by birds using a Marantz PMD 660 (2012-2013)/661 (2014) solid state digital recorder attached to a Sennheiser ME62 omnidirectional microphone and a Mineroff parabolic reflector. The sex of the responding birds was easy to determine using color bands and sex-specific vocalizations.

For all conspecific trials, we played a stimulus composed of song recordings from a single individual, separated by 5 seconds of silence, filtered at 1 kHz, standardized to the same peak amplitude (1.4 kU) in Raven Pro v1.4 (Bioacoustics Research Program 2011), and played back at a peak amplitude of 70-75 dB as measured by an Extech digital sound level meter (model 407732) at 1 m. As part of another study, each bird also heard a heterospecific playback trial (for details see Chapter 1 “*Playback Experiment*”, Chapter 4 “*Appendix A*”). Each bird heard a

randomly selected stimulus from the set being used that year (female trials: 6 stimuli in 2012, 15 in 2013, 9 in 2014; male trials: 6 stimuli in 2012, 14 in 2013, 8 in 2014; heterospecific trials: 6 stimuli all years). We never tested birds with their own song, song of an immediate neighbor, or songs to which they had been exposed in a previous year. Each bird was tested with one trial per morning (sunrise to 12:30 EST) within the first four days of the female's egg laying period until all three trial types were completed. We randomized trial order. Exposure to opposite-sex playback did not alter male or female aggressive responses to subsequent same-sex trial (for details see Chapter 4 "*Appendix A*").

We were concerned that having a model present during the pre-playback period of the conspecific trials but not the heterospecific trials may produce systematic differences. Therefore, in 2014, we replaced the wren model with a wooden egg of roughly the same size and color which we presented during all trial types. Aggressive responses did not differ between model types, suggesting birds primarily respond to acoustic playback stimuli (Mann-Whitney tests; female during heterospecific trial: $P = 0.69$; male during heterospecific trial: $P = 0.86$; female during female trial: $P = 0.69$; male during male trial: $P = 0.66$). Year is included as a covariate in all analyses involving the egg model year.

We calculated each bird's aggression score using the formula below (Chapter 2 "*Aggression Score*").

Aggression score = $3 * (\# \text{ of attacks}) + 2 * (\# \text{ of dives}) + 1 * ((300 \text{ sec} - \text{sec to approach within } 5\text{m}) / 300 \text{ sec}) + 1 * ((\text{sec within } 1\text{m during playback} - \text{sec within } 1\text{m during pre-play}) / 300 \text{ sec})$

From this point forward, “female aggressiveness” and “male aggressiveness” refers to responses in the same-sex trials. We considered a dive to be a rapid movement passing within several centimeters of the model or speaker. We considered attacks to be any physical contact with the model or the speaker. If the bird was never seen or heard during the entire 15 minute trial, we dropped them from further analysis. For birds with multiple mates in the season, we only examined the first test of each trial type.

Mate Quality

To determine whether female aggression helped females obtain higher quality mates, we examined four variables that might indicate male quality: male feeding effort, male mass, male condition, and male aggression score. To measure male feeding effort, we observed nests twice when the nestlings were between 5-8 days old in both 2012 and 2013. After a 5 minute habituation period, a human observer sitting quietly approximately 30 m from the nestbox recorded how many times the male and female entered the box with a food item for 25 minutes. The male and female were easily distinguished using unique color bands. If the pair was still visibly disturbed by the human observer after 5 minutes, we stopped observing. We averaged male and female feeding rates for the two observations to give one measurement of feeding effort for each individual.

We used linear models to determine whether aggressive females had high quality mates. We used a model with female feeds per offspring included as a covariate to determine whether aggressive females had mates that feed more per offspring ($N = 54$ females, 2012 and 2013). We used models with year included as a covariate to determine whether aggressive females had mates that were heavier ($N = 144$), in better condition ($N = 144$), or more aggressive ($N = 163$) in all years between 2012 and 2014. Some females had more than one mate per season. We

included all mating pairs in this analysis. We log transformed male and female aggression scores to meet model assumptions.

Female Investment in Reproduction

To determine whether female aggression affected maternal investment in egg production, we measured each egg to the nearest 0.1 g on the day it was laid in all years between 2012 and 2014. We used linear models with female mass and year included as a covariate to determine whether female aggression affected the average mass and total number of eggs a female laid over the season ($N = 94$). Although total numbers of eggs were count data, this model met all assumptions of a linear model.

Parental Behavior and Offspring Performance

Female Parental Behavior. To determine whether female aggression affected female feeding rate, we used a linear model with male feeds per offspring included as a covariate ($N = 65$, 2012 and 2013). We measured the number of female feeds per offspring as described above (under “*Male Quality*”). Female aggression was log transformed to meet model assumptions.

Brood Enhancement. To determine whether more aggressive females had a more difficult time coping with increased reproductive demands, we experimentally enhanced the size of 31 clutches in 2013 and 7 clutches in 2014. For 17 clutches in 2013 and all 7 clutches in 2014, we gave nests 1 to 4 additional eggs depending on how many eggs were left from birds that were experimentally evicted as part of another experiment (for details on the eviction experiment, see Chapter 4, “*Eviction Experiment*”). In 2013, we gave an additional 14 clutches either 2 or 3 eggs from a randomly selected clutch that was initiated at the same time. Some experimentally enhanced clutches subsequently lost eggs to house wren ovicide, predation, human error or infertility. We only considered clutches enhanced for the purposes of this study

if more than 7 eggs ultimately hatched. Unmanipulated house wren clutches averaged 6.32 ± 0.98 (SD) eggs between 2012 and 2014.

Offspring Size. To determine whether female aggression influenced offspring size, we measured the mass of nestlings 4, 6, 8, and 10 days after hatching for all nests between 2012 and 2014. In all analyses, we took the average of all nestlings in the nest at each time point. We used linear models with female aggression, male aggression, female mass, male mass, year, and day in the season included as covariates (day 4 $N = 87$ nests, day 6 $N = 84$ nests, day 8 $N = 78$ nests, day 10 $N = 73$ nests). We also included an interaction between female aggression and brood enhancement treatment to determine whether the relationship between female aggression and nestling size changes when broods are experimentally enhanced. Male and female aggression scores were log transformed to meet model assumptions. We attempted an unsuccessful cross-foster experiment on a small number of broods in 2014 (16 out of 52 broods). We dropped all the cross-fostered brood from this analysis of offspring mass, although inclusion of these broods did not change the significance of the main effects reported here.

Annual Fledging Success

To determine whether female aggression had an effect on offspring survival, we measured the proportion of nestlings that eventually fledged for each female in each year between 2012 and 2014. Offspring losses at the nestling stage were due to predation (74% of losses), starvation and/or exposure (14% of losses) or the death/disappearance of the female (6% of losses). We used a binomial generalized linear model with year and the average territory preference score of the territories each female occupied across the season included as a covariate, as territory preference score independently affects the probability of fledging (see Appendix). Binomial generalized linear models are appropriate for proportional data that can be considered a

series of “successes” and “failures” (Bolker 2008). We also examined the proportion of eggs that resulted in fledging offspring using the same model, thus capturing the competition effects reported in Chapter 4. Any females that experienced eviction, brood reduction or brood enhancement during the season were dropped from the data set (71 females in the final data set). We log transformed female aggression to meet model assumptions.

Female Survival

To determine whether aggression affects survival of adult females, we compared the aggression scores of the females that returned to the study site the next year to the aggression scores of females that did not return. Overall returns rates were low (15 females from 2012, 3 females from 2013). We used a binomial generalized linear model with year included as a covariate to test the effect of aggression score on whether a female did or did not return ($N = 83$ females, 18 returned). We also ran a simpler binomial generalized linear model using whether a female escalated to physical aggression instead of aggression score.

Statistical Note

We performed all statistical tests in R version 3.0.2 (R Core Team 2013; package ‘car’: Fox & Weisberg 2011; package ‘MuMIn’: Barton 2015). We assessed all linear and gamma generalized linear models for heteroscedasticity and normality of residuals using model diagnostic plots. We assessed all models for outliers using Cook’s distance and multicollinearity using VIF values (cutoff VIF value of 4, all VIF values were less than 1.5). Because binomial generalized linear models do not produce the R^2 values used in traditional linear models, we instead calculated goodness-of-fit with pseudo- R^2 values based on a likelihood ratio test (Magee 1990) using the “r.squaredLR” command in the package “MuMIn” (Barton 2015).

Ethical Note

This research was approved by the Michigan State University's Institutional Animal Care and Use (ACUC) panel. Field work was conducted under appropriate federal and state permits (MSU IACUC Animal Use Form numbers 04/11/076-00, 03/14-062-00; U. S. Fish and Wildlife Service Federal Banding Permit number 23587; Michigan Department of Natural Resources Scientific Collectors Permit number SC 1432, U. S. Fish and Wildlife Service Permit number MB01550B-1). No females abandoned territories following our tests. No birds suffered adverse effects during capture and measurement.

RESULTS

Mate Quality

Female aggression had no detectable relationship with various measures of male mate quality. Mates of more aggressive females did not feed offspring more often after controlling for the significant negative effect of female feeding rate (linear model, $N = 54$, $F = 1.03$, $P = 0.31$, model $R^2 = 0.07$). More aggressive females did not have mates that were heavier (linear model, $N = 144$, $F = 0.390$, $P = 0.53$, model $R^2 = 0.09$) or in better condition (linear model, $N = 144$, $F = 0.330$, $P = 0.57$, model $R^2 = 0.1$) after controlling for the significant effect of year. Finally, more aggressive females were not mated to more aggressive males after controlling for the significant effect of year (linear model, $N = 163$, $F = 1.717$, $P = 0.19$, model $R^2 = 0.09$).

Female Investment in Reproduction

Aggressive female house wrens did not invest less in eggs. Female aggression had no effect on egg mass after controlling for the significant positive effect of female mass (linear model, $N = 94$, $F = 1.065$, $P = 0.30$, model $R^2 = 0.06$). Aggressive females did not lay fewer

eggs than their less aggressive counterparts after controlling for the effect of female mass (linear model, $N = 94$, $F = 0.626$, $P = 0.43$, model $R^2 = 0.02$).

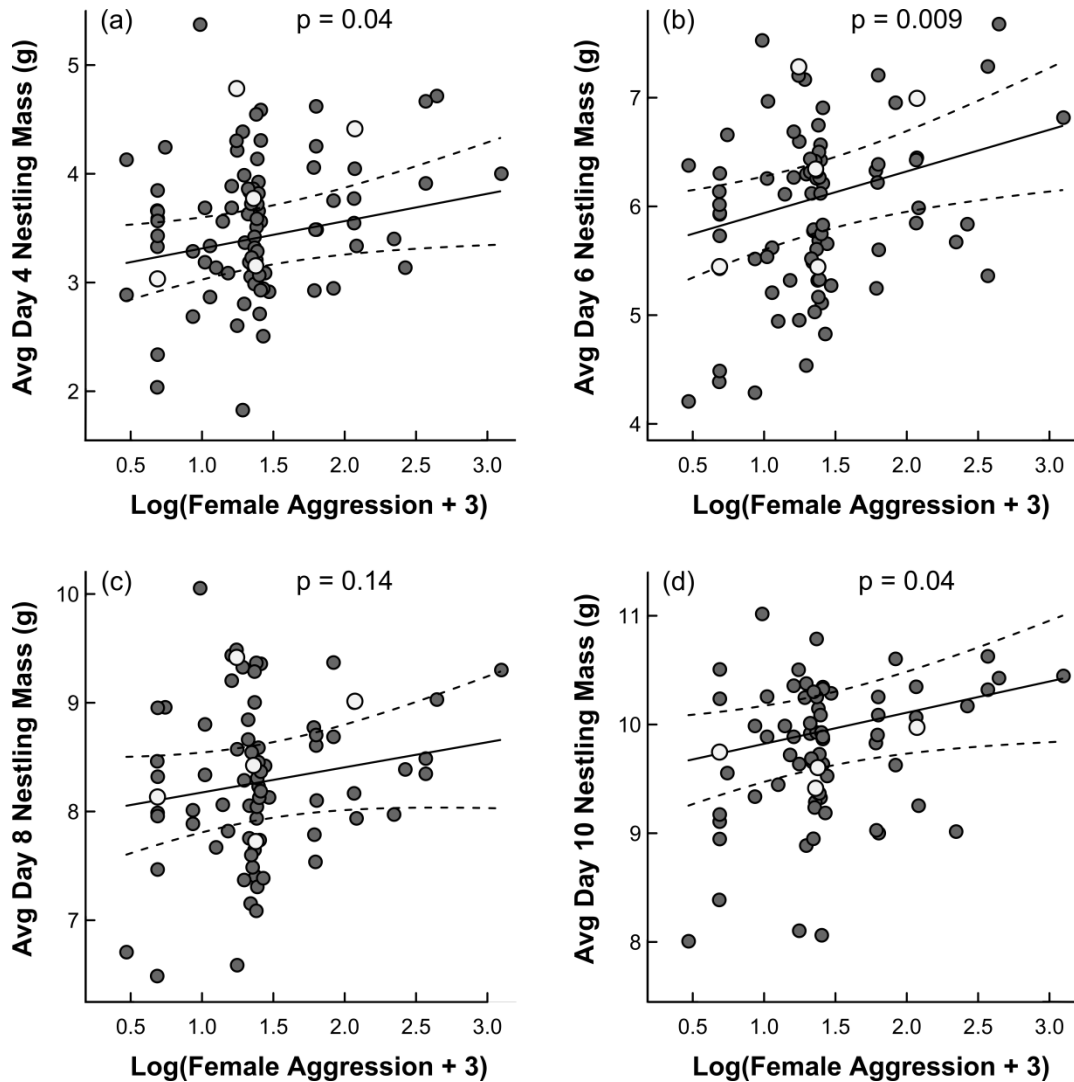


Figure 5.1: The effect of female aggression on average nestling mass on (a) day 4 ($N = 87$ nests), (b) 6 day ($N = 84$ nests), (c) day 8 ($N = 78$ nests), and (d) day 10 ($N = 73$ nests) after hatching. White points represent broods that were experimentally enhanced in 2013 or 2014. Lines are predicted values from linear models $\pm$ 95% CIs. Each point represents the average of all offspring within the nest.

Table 5.1: Results from linear models on the size of house wren nestlings on day 4, 6, 8, and 10 after hatching. Bolded variables have $P < 0.05$.

Variable	Day 4 model ($N = 87$, $R^2 = 0.23$)			Day 6 model ($N = 84$, $R^2 = 0.28$)		
	Effect Size $\pm$ SE	F	P	Effect Size $\pm$ SE	F	P
Year		2.141	0.12		0.447	0.64
Female aggression	0.252 ± 0.120	4.371	0.04	0.383 ± 0.142	7.284	0.009
Male aggression	0.107 ± 0.085	1.575	0.21	0.173 ± 0.102	2.885	0.094
Female mass	0.008 ± 0.083	0.009	0.93	-0.037 ± 0.099	0.137	0.71
Male mass	0.052 ± 0.125	0.171	0.68	0.300 ± 0.156	3.709	0.06
Day in season	-0.010 ± 0.003	12.981	0.0006	-0.013 ± 0.003	14.567	0.0003
Female aggression: brood enhancement	0.266 ± 0.207	1.639	0.20	0.192 ± 0.244	0.616	0.44
Variable	Day 8 model ($N = 78$, $R^2 = 0.17$)			Day 10 model ($N = 73$, $R^2 = 0.15$)		
	Effect Size $\pm$ SE	F	P	Effect Size $\pm$ SE	F	P
Year		0.127	0.88		0.681	0.51
Female aggression	0.231 ± 0.155	2.219	0.14	0.287 ± 0.137	4.382	0.04
Male aggression	0.175 ± 0.110	2.562	0.11	0.106 ± 0.098	1.170	0.28
Female mass	0.139 ± 0.113	1.512	0.22	0.057 ± 0.105	0.292	0.59
Male mass	0.144 ± 0.173	0.688	0.41	0.234 ± 0.159	2.179	0.14
Day in season	-0.013 ± 0.004	12.890	0.0006	-0.009 ± 0.003	6.927	0.01
Female aggression: brood enhancement	0.050 ± 0.252	0.039	0.84	-0.202 ± 0.246	0.677	0.41

Parental Behavior and Offspring Performance

The rate at which females fed their offspring was not affected by female aggressiveness (linear model, $N = 65$, $F = 0.0008$, $P = 0.98$, model $R^2 = 0.03$) after controlling for male feeding behavior. Nevertheless, more aggressive females were more likely to have larger offspring (Figure 5.1). Female aggression was significantly related to offspring size on nestling days 4, 6, and 10 (day 4: $N = 87$ nests, $P = 0.04$; day 6: $N = 84$ nests, $P = 0.009$, day 10: $N = 73$ nests, $P = 0.04$, see Table 5.1 for additional model details), after controlling for male aggression, parental mass, year, and day in season. Although the effect of female aggression was not significant on nestling day 8, the trend was in the same direction as on other days (Table 5.1, $N = 78$ nests, $P =$

0.14). The effect of female aggression was strongest on nestling day 6, which is during the period when nestlings grow most rapidly (Figure 5.2). In contrast to female aggression, male aggression had no detectable relationship with offspring size at any time point (Table 5.1).

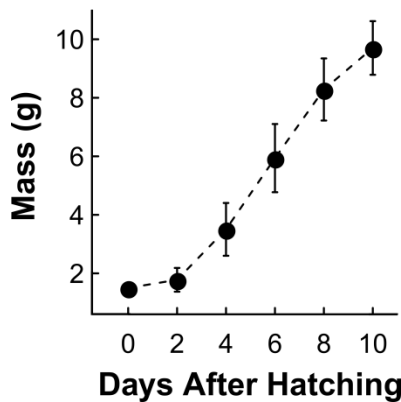


Figure 5.2: Growth of house wren nestlings between 2012 and 2014. Bars represent SD.

The positive effect of female aggression on nestling size persisted when we experimentally enhanced brood size. The interaction between female aggression and brood enhancement treatment was not significant in any model (see Table 5.1). Furthermore, the positive effect of female aggression on offspring size did not appear to be due to a correlation between female aggression and female size. Female mass did not significantly affect nestling size at any time point (Table 5.1). We have previously shown that larger females are not more aggressive than smaller ones (see Chapter 3, Table 3.1).

Annual Fledging Success

Aggressive females were more successful at completing the reproductive attempts they began. More aggressive females had a higher probability of raising their nestlings to fledging, even after controlling for the fact that more desirable territories had higher fledging success (Figure 5.3a; binomial generalized linear model; $N = 71$ females, female aggression: estimate $\pm$ SE = 1.41 ± 0.3 , $\chi^2 = 23.664$, $P < 0.0001$; territory preference score: estimate $\pm$ SE = $-0.021 \pm$

0.009, $\chi^2 = 5.560$, $P = 0.02$; model likelihood-ratio pseudo- $R^2 = 0.57$). Year was also significant in this model (year: $\chi^2 = 34.237$, $P < 0.0001$). We have previously determined that more aggressive females are more successful at protecting eggs from house wren ovicide (Chapter 4, Figure 4.3). Overall, eggs laid by more aggressive females were more likely to lead to nestlings that fledged successfully (Figure 5.3b; binomial generalized linear model; $N = 71$ females, estimate $\pm$ SE = 0.574 ± 0.188 , $\chi^2 = 9.658$, $P = 0.002$; territory preference score: estimate $\pm$ SE = -0.03 ± 0.007 , $\chi^2 = 22.373$, $P < 0.001$; year: $\chi^2 = 70.590$, $P < 0.001$; model likelihood-ratio pseudo- $R^2 = 0.81$).

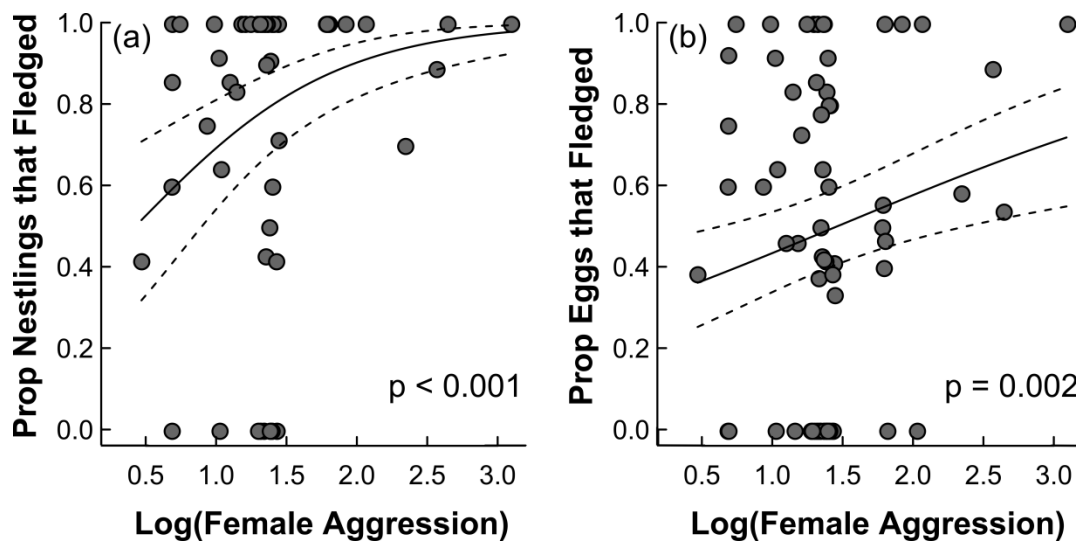


Figure 5.3: The effect of female aggression score on the proportion of nestlings that successfully fledged (a) and the proportion of eggs leading to fledged offspring (b) between 2012 and 2014 ($N = 71$ females). Lines represent predicted values from binomial generalized linear models $\pm$ 95% CIs.

Female Survival

More aggressive females may be less likely to survive between years. Although the effect we observed was not significant, females that returned to the study site the next year tended to have lower aggression scores than the ones that failed to return after controlling for the

significant effect of year (Figure 5.4a; binomial generalized linear model: $N = 83$, aggression score: estimate $\pm$ SE = -0.195 ± 0.158 , $\chi^2 = 2.447$, $P = 0.118$, model likelihood ratio pseudo- $R^2 = 0.21$). Few birds returned in 2014 and overall the population size was small that year. Females that ultimately returned were significantly less likely than others to escalate all the way to physical aggression during simulations of female intruders after controlling for the significant effect of year (Figure 5.4b; binomial generalized linear model: $N = 83$, physical aggression: estimate $\pm$ SE = -1.817 ± 1.101 , $\chi^2 = 3.949$, $P = 0.047$, likelihood ratio pseudo- $R^2 = 0.24$). Only one female that escalated to physical aggression returned the following year. It should be noted, however, that this difference does not directly indicate a difference in survival. Although a female may not have returned to our study site, she might have survived but moved to another location.

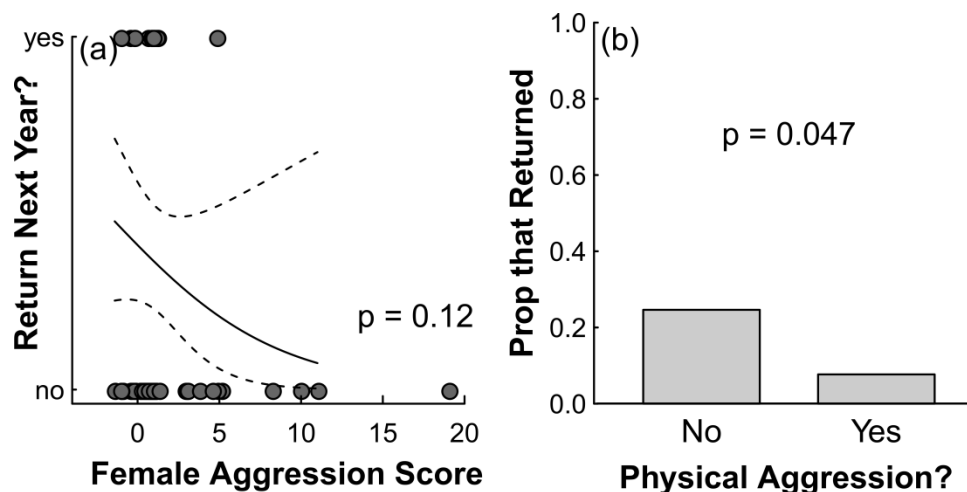


Figure 5.4: (a) The aggression score of female house wrens that did and did not return to the study site in the subsequent year. Lines represent simulated values from a binomial generalized linear model $\pm$ 95% CIs ($N = 83$ females). (b) The proportion of females that returned based on whether they did or did not dive at or attack the simulated female intruder.

DISCUSSION

In males, aggressiveness is one aspect of a phenotype that affects various components of male fitness. Here we found that female aggressiveness toward same-sex intruders has a variety of fitness benefits throughout the breeding season. Although more aggressive female house wrens did not mate with higher quality males, they had larger offspring and successfully raised a larger proportion of these offspring to fledging. In other songbirds, offspring that are larger at the time of fledging are more likely to survive to the next year (Gebhardt-Henrich & Richner 1998). Contrary to some theoretical predictions (Fitzpatrick et al. 1995, Chenoweth et al. 2006), aggressive female house wrens did not invest fewer resources in egg production. We have previously shown that more aggressive females are more successful at gaining access to nesting cavities and protecting their clutches from conspecifics when competition is experimentally elevated (Chapter 4). Instead of helping females gain access to quality mates, same-sex aggression in female house wrens provides females with direct benefits when it comes to resource competition and reproductive performance. While aggressive females are more successful within a season, our data suggest that aggressive females might be less likely to survive between breeding seasons.

Female Aggression: Resources Trump Mate Quality

Female house wrens do not appear to compete aggressively with other females for access to high quality mates. In previous work, we have documented that female house wrens become more aggressive when they face the biggest risk of losing their nesting cavity but not when they pair with mates they perceive as higher quality (Chapter 3). Although previous work in this population showed that females made subtle adjustments in reproductive investment based on perceived mate quality (Dubois et al. 2006), female-female aggressive competition in house

wrens may be more important with respect to gaining and keeping territories (Chapter 4) or ensuring that males remain monogamous (Liker & Székely 1997, Sandell 1998, Slagsvold & Lifjeld 1994, Veiga 1992). In other species, female competition more commonly revolves around access to food, resources like nest sites, or proxies for priority of resource access, like social rank, rather than access to mating partners (reviewed in Berglund et al. 1993, Clutton-Brock 2009, Clutton-Brock & Huchard 2013, Rosvall 2011b, Stockley & Bro-Jørgensen 2011, Stockley & Campbell 2013, Tobias et al. 2012). When females do compete for access to particular males, they often do so under circumstances of male sperm limitation or conditions where direct benefits bestowed by males, like spermatophores, profoundly affect female reproductive capacity (Bro-Jørgensen 2011, Gwynne & Simmons 1990, Rosvall 2011b). Male house wrens primarily provide females with feeding assistance during the nestling period, aid in defense of the nest, and genes that may affect offspring success (Johnson et al. 1993, Johnson & Albrecht 1993). However, female house wrens can sometimes raise offspring with no or very little assistance from males (Johnson et al. 1992, Johnson et al. 1993, Johnson et al. 1994). Our findings that more aggressive females were not mated to males that were bigger, in better condition, or more likely to feed offspring more suggest females compete with other females for resources unrelated to mate quality. Nevertheless, there might be additional dimensions of male quality we have not captured in this study.

Do Aggressive Females Make Better Mothers?

Theory predicts and empirical studies suggest that investing resources, time, and energy in aggressive competition should decrease a female's their ability to invest in offspring production and care (Bell et al. 2012, Chenoweth et al. 2006, Fitzpatrick et al. 1995, Packer et al. 1995, Rosvall 2011a, Trivers 1972, but see Simmons & Emlen 2008). This hypothesis is often

invoked to explain why female aggression is generally less intense than male aggression within species (Clutton-Brock & Huchard 2013, Stockley & Campbell 2013). Contrary to this prediction, we found that aggressive females had bigger offspring, were more likely to raise those offspring to fledging, and did not differ in their provisioning rates from less aggressive females. This contrasts with patterns found in males and females in some other species (O'Neal et al. 2008, Rosvall 2011a, Rosvall 2013b, Cain & Ketterson 2013a, McGlothlin et al. 2007, Tuttle 2003, Pryke & Griffith 2009 but see Cain & Ketterson 2012, Jawor et al. 2004).

The reproductive trade-off hypothesis is typically invoked for males in species where intrasexual aggression during the breeding season provides just one benefit: enhanced access to mates (Magrath & Komdeur 2003, Stiver & Alonzo 2009). However, females often compete for resources that can have important effects on fecundity and offspring care (Cain & Ketterson 2013a, Cain & Rosvall 2014, Tobias et al. 2012). Aggressive females may be able to compensate for any decreases in fecundity due to competition by winning access to higher quality resources for offspring production (Cain & Ketterson 2013a, Hodge et al. 2008, Packer et al. 1995, Watson & Simmons 2010). Furthermore, if females face intense competition for access to resources required to breed at all (e.g. nest cavities), selection should favor female aggressiveness. In theory, a less aggressive female might be able to invest more in reproduction, but, in practice, she would be excluded from breeding by more aggressive counterparts. This is the same logic used to explain how traits expressed during male mating competition can evolve even in the face of survival costs (Darwin 1871, Shuster & Wade 2003). Additionally, if aggression in one context (e.g. same-sex competition) is correlated with aggression in other contexts (e.g. defense against predators), these benefits could overwhelm any potential fecundity costs (Cain et al. 2011). Predation is a common cause of nestling loss in house wrens (Krieg

unpublished data, Belles-Isles & Picman 1986). If more aggressive house wrens react more aggressively to predators as well as to conspecifics, this might explain why aggressive females are more successful at fledging offspring. Complex relationships between aggression and reproductive performance may indeed be the norm for females in many species.

There are several possible reasons why more aggressive female house wrens had bigger babies. Aggressive female house wrens might have had higher concentrations of circulating testosterone than less aggressive counterparts. In some species, females with higher concentrations of circulating testosterone lay eggs with higher levels of maternally deposited androgens (Jawor et al. 2007, Rutkowska et al. 2005, Schwabl 1996a, Whittingham & Schwabl 2002). Nestlings that hatch from eggs with naturally or experimentally elevated androgens grow faster in some species (Groothuis et al. 2005a, Groothuis et al. 2005b, Schwabl 1996b but see Barnett et al. 2011, Clairardin et al. 2011, Sandell et al. 2009, Sockman & Schwabl 2000). Hormone sampling or experimental manipulations would be needed to determine whether aggression among female house wrens is mediated by testosterone or other androgens. Alternatively, aggressive females may spend more time incubating, which we did not measure in this study. Offspring that experience warmer temperatures during embryonic development grow larger (Ardia et al. 2010), although other studies have found that more aggressive females actually incubate less (Cain & Ketterson 2013b, Rosvall 2013b). On the other hand, aggressive female house wrens may be better at finding or exploiting high quality food sources for offspring provisioning, even though they did not provision more frequently by our measure. Aggressive females might also have provisioned eggs with additional resources that were not captured in our simple measure of egg mass. Finally, aggressive females might be of higher quality in some dimension not captured by condition or mass (see Chapter 3), which allows them to produce

higher quality offspring despite an intrinsic trade-off between reproduction and competitive ability (Magrath & Komdeur 2003, Stearns 1992).

Future Directions

Understanding the proximate mechanisms underlying female aggression may help make sense of the reproductive pattern we document here. Many of the costs to male aggression (decreased parental performance, depressed immune function, etc.) are mediated by testosterone (Hau 2007, Ketterson et al. 1992, Wingfield et al. 2001). However, in species where male parental care is critical to offspring survival, males may evolve behavioral insensitivity to testosterone (Lynn 2008, Wingfield et al. 2001). In some of these species, males mediate aggression using alternative mechanisms (Schlinger & Ramage-Healey 2011, Soma et al. 2008, Trainor et al. 2006, Wingfield 2012). In a growing number of species, researchers have found that differences in female aggressiveness are explained by alternative circulating hormones (e.g. progesterone, estradiol) or differences in neural sensitivity to steroids, rather than differences in circulating testosterone (Goymann et al. 2008, Pärn et al. 2008, Rosvall et al. 2012, Woodley & Moore 1999). These alternative mechanisms are predicted to evolve in species in which competition for breeding resources and maternal care occur during the same time periods because of the suppressive effect of testosterone on parental behavior (Rosvall 2013a). Female house wrens may mediate aggression through an alternative mechanism to testosterone in order to avoid the suppressive effect on parental performance.

Female aggression may also carry longer term costs for female house wrens. Our data suggests that more aggressive females may be less likely to return from migration the next year, although without more study we cannot be sure whether this reflects a true difference in survival or a difference in philopatry. In some species, more aggressive males have depressed immune

function (Casto et al. 2001, Folstad & Karter 1992, Greives et al. 2006, Wingfield et al. 2001). Aggressive females may suffer costs like these, which are not immediately apparent when assessing reproductive performance within a single year.

Conclusions

Intrasexual aggression among female house wrens clearly has important fitness consequences. We have previously shown that female aggression helps females gain and keep access to valuable nesting cavities. Here we show that aggressive females raise larger offspring (a common proxy for offspring quality, Rosvall 2011a) and successfully fledge a larger proportion of offspring. If aggression is heritable in female house wrens, intrasexual aggressiveness may be under selection. These patterns do not match patterns found in males in some other species. Female aggression in house wrens does not appear to play a role in competition for high quality males. Additionally, female aggression does not appear to negatively affect parental performance. Whereas certain benefits (e.g. winning nesting cavities) are likely shared between the sexes, other benefits (e.g. enhanced offspring size) appear unique to females. This work highlights the importance of explicitly considering the contexts in which females compete and the resources that affect female fitness when studying intrasexual female aggression (Stockley & Campbell 2013, Cain & Rosvall 2014).

CHAPTER 5 ACKNOWLEDGEMENTS

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APPENDIX

APPENDIX

Assessing territory preference

House wren territories at our study site vary in vegetation structure and the number of boundaries they share with other house wren territories. Between years, the same territories were more likely to be occupied than others. To determine which territories were more desirable, we determined the day on which the first egg was laid on each territory, as this variable was reliably measured in the historical data set. The date on which the first egg of the entire season was laid was set as day one and the preference index of all other territories was measured relative to this date (e.g. territory 1 first egg date: May 8th, score = 1; territory 2 first egg date: May 11th, score = 4). We gave territories that were never occupied a score of 100, marking the end of the season. We then averaged the territory preference scores between 2008 to 2012 and 2014 to obtain an average preference score for each territory. 2013 was omitted because the population-wide manipulation of competition likely altered patterns of territory settlement (see Chapter 4 for details).

Territories with an earlier territory settlement in one year were likely to be settled earlier in the next year (Figure 5.5). Here we assume that early settlement indicates the territory was more “desired”. In years when the population size was small (2011 and 2014), this correlation was weaker but still significant. In all pair-wise comparisons between years, the correlation between preference scores were positive (Table 5.2; Pearson’s r : 0.2 to 0.63), with one exception (2008 and 2012, Pearson’s r = -0.02). This pattern was not simply driven by the same females

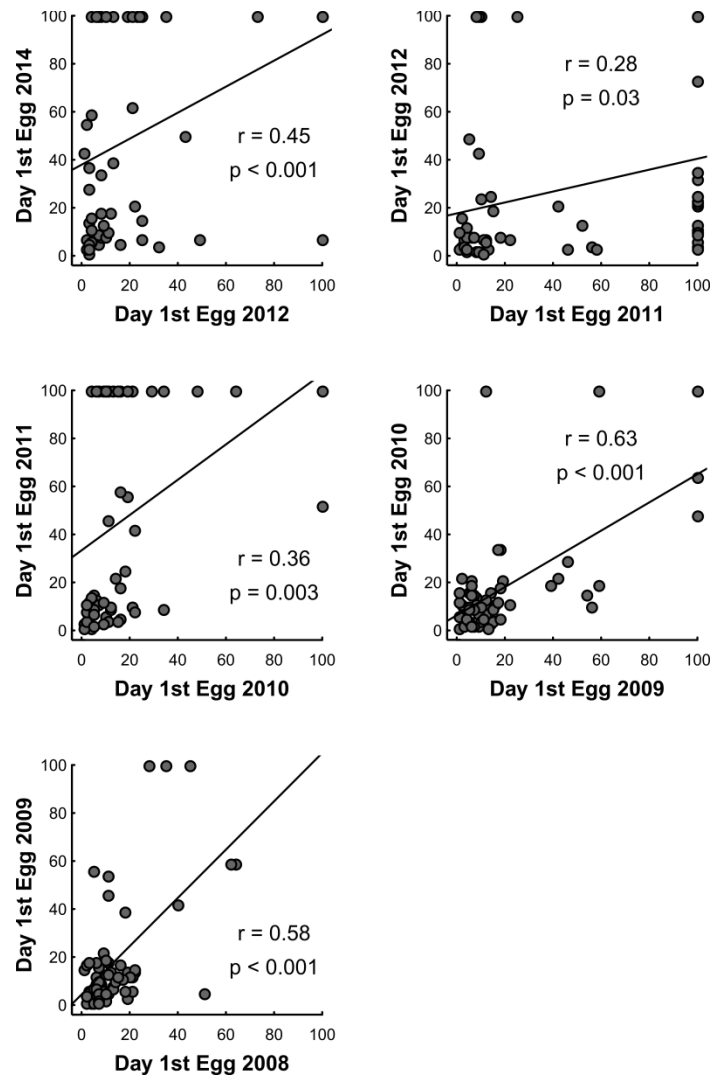


Figure 5.5: The relationship between the day on which the 1st egg was laid on each territory in 2014 (33 females), 2012 (56 females), 2011 (47 females), 2010 (66 females), 2009 (64 females), and 2008 (69 females). R indicates Pearson's correlation coefficients. Territories that were not settled that year were given a score of 100, marking the end of the season.

returning to the same territories year after year. While a portion of returning females do return to the same locations (0% - 52.6% of returning females each year, mean = 28.7%), the majority of the population every year is composed of unbanded females who were born elsewhere and had not previously bred at the study site (between 59.4% - 80.9% of females in the population between 2008 and 2014; mean = 69.8%). The positive correlation between years suggests that females use some of the same cues when deciding where to settle. The fact this correlation was

not perfect between years is also unsurprising. Females likely vary in the information they have about the study area, their breeding experience, and territory conditions may interact with seasonal weather conditions. Variables that make one territory more desirable than another need further investigation.

Table 5.2: The Pearson's correlation coefficients for the day of the first egg on each territory from 2008-2012 and 2014 (^{***} $P < 0.001$, ^{**} $0.001 < P < 0.01$, ^{*} $0.01 < P < 0.05$, [^] $0.05 < P < 0.1$). Bolded values indicate $P < 0.05$

Year	2008	2009	2010	2011	2012	2014
2008	-	0.58^{***}	0.47^{***}	0.24 [^]	-0.02	0.28[*]
2009	-	-	0.63^{***}	0.42^{***}	0.23 [^]	0.28[*]
2010	-	-	-	0.36^{***}	0.20	0.28[*]
2011	-	-	-	-	0.28[*]	0.32^{**}
2012	-	-	-	-	-	0.45^{***}
2014	-	-	-	-	-	-

Nevertheless, territories that were consistently settled earlier and had lower territory preference scores appeared objectively better using several measures. Between 2008 and 2012, females cumulatively laid significantly more eggs on more preferred territories (Figure 5.6a, linear model, $N = 59$ territories, estimate $\pm$ SE = -0.738 ± 0.059 , $F = 154.08$, $P < 0.0001$, $R^2 = 0.73$). We did not include 2013 and 2014 because of experimental manipulations of competition and clutch sizes. More preferred territories also cumulatively produced significantly more fledging nestlings between 2009 and 2012 (Figure 5.6b, linear model, $N = 59$ territories, estimate $\pm$ SE = -0.341 ± 0.051 , $F = 44.271$, $P < 0.0001$, $R^2 = 0.43$). We omitted data from 2008 because

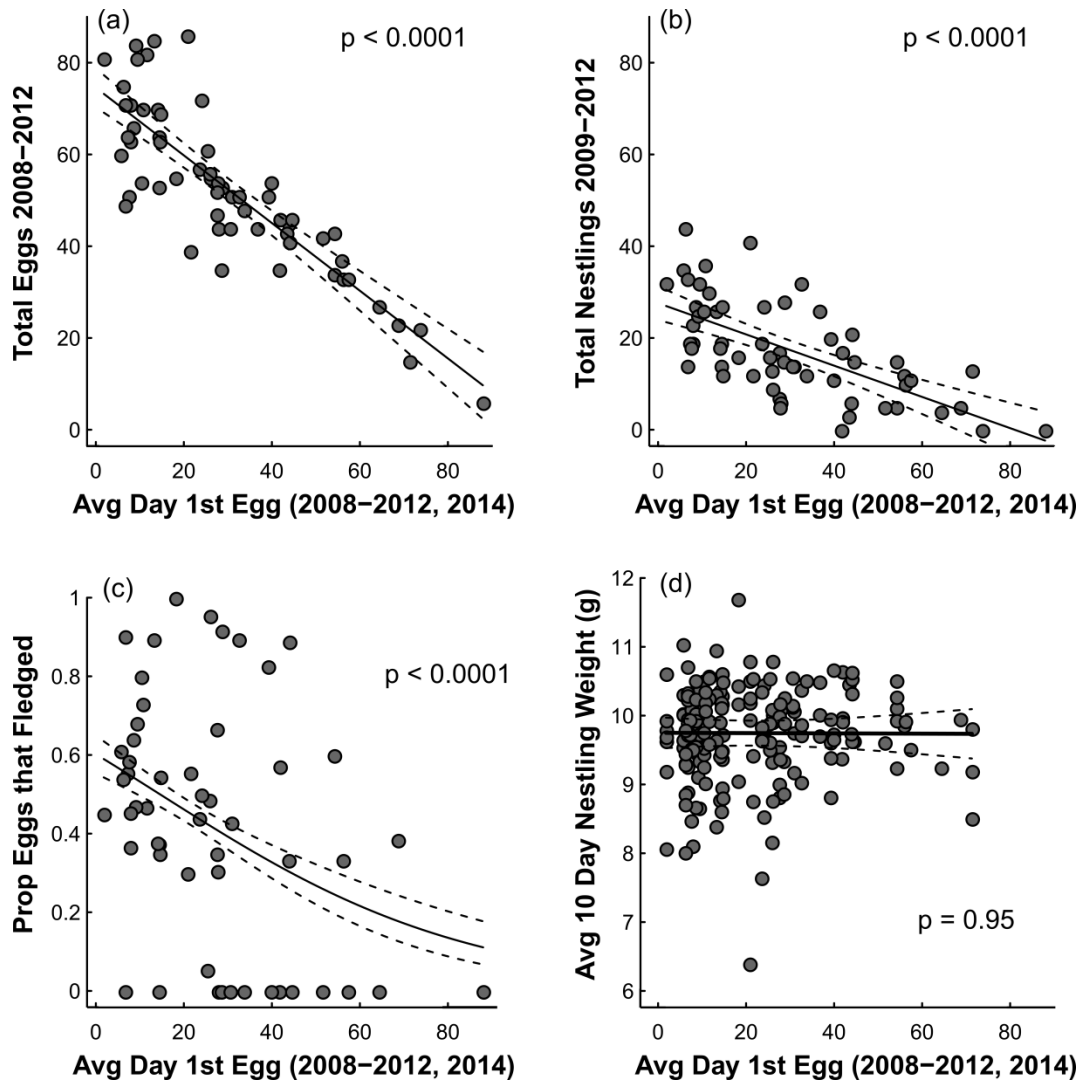


Figure 5.6: The relationship between the average day of the first egg on each territory and (a) the total number of eggs produced on that territory from 2008 to 2012, (b) the total number of nestling fledged from that territory between 2009 and 2012, (c) the proportion of eggs produced on that territory that ultimately produced a fledging offspring (2012-2014), and (d) the average size of nestlings at day 10 (2009 to 2014). Lines represent predicted values $\pm$ 95% CI from linear models in (a), (b), and (d) and a binomial generalized linear model in (c). Points in (a), (b), and (c) represent territories ($N = 59$ in a and b, 58 in c). Points in (d) represent nests ($N = 189$).

experimental injections of testosterone into eggs performed by another researcher (J. Johnson) may have affected hatching success. A higher proportion of eggs laid on preferred territories between 2012 and 2014 ultimately resulted in a nestling that fledged (Figure 5.6c, binomial

generalized linear model, $N = 58$ territories, estimate $\pm$ SE = -0.028 ± 0.004 , $\chi^2 = 57.483$, $P < 0.0001$, pseudo- $R^2 = 0.66$). We removed one territory that was an outlier using Cook's distance, however, including this point did not change the significance of the conclusions. Detailed records of egg and nestling losses were not kept from 2008 to 2011. Any eggs that did not reach fledging due to the eviction manipulation in 2013 were not included in these data (see Chapter 4). Although preferred territories did produce more nestlings, territory preference index did not affect the mass of nestlings on day 10 between 2009 and 2014 after controlling for the effects of year and day in the season (Figure 5.6d, linear model; $N = 189$ nests, territory preference index: $F = 0.004$, $P = 0.95$; year: $F = 1.613$, $P = 0.17$; day in season: $F = 12.925$, $P = 0.0004$; model $R^2 = 0.06$). We did not have complete nestling size records for 2008. Reproductive success may be higher on preferred territories due to differences in predator density, differences in vegetation structure around the nest that alter the probability of predation, or differences in food resources, although this last option is less likely because nestling size did not vary based on territory preference score (Figure 5.6d). These patterns appear to be driven by characteristics of the territory, not characteristics of the females that settle on these territories. More aggressive or better condition females do not have territories with better preference scores after controlling for the significant effect of year (gamma generalized linear model, $N = 103$ females, log transformed female aggression score: $\chi^2 = 0.0009$, $P = 0.98$; female condition: $\chi^2 = 0.120$, $P = 0.73$; year: $\chi^2 = 7.547$, $P = 0.02$).

Birds also behave as though territories that are settled consistently earlier are more preferable. House wrens will sometimes destroy other house wren eggs when attempting to usurp a nestbox. On some occasions, intruders will destroy only a few eggs before being successfully repelled by the territory owners. When a male or female intruder permanently

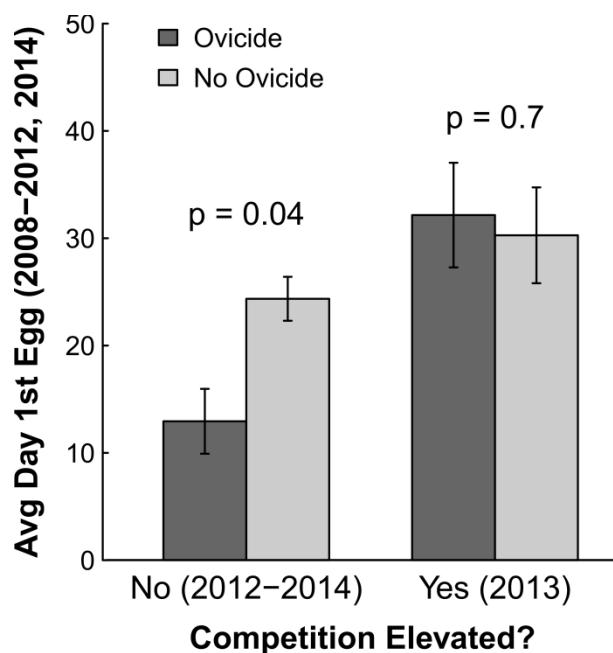


Figure 5.7: The mean $\pm$ SEM territory preference score for nests that did and did not experience house wren ovicide in the years competition was not manipulated (2012, 2014, $N = 79$) compared to the year competition was experimentally enhanced (2013, $N = 41$).

replaces the original territory owner in our population, they always destroy the entire clutch (personal observation). Therefore, house wren ovicide losses can be used as an indirect measure of intruder pressure. In the years that competition was not experimentally elevated (2012 and 2014), territories that lost at least one egg to house wren ovicide had significantly lower territory preference scores (i.e. more desirable) than territories that did not experience ovicide (Figure 5.7; Mann-Whitney U test, $W = 184.5$, $P = 0.045$). In the year that competition was elevated (2013), ovicide rates were higher (see Chapter 4, Figure 4.2) and territories that experience ovicide did not differ in preference score from territories that did not experience ovicide, possibly because competition over territories resources was so elevated that birds attempted takeovers on high and low quality territories (Mann-Whitney U test: $W = 224$, $P = 0.70$). Ovicide losses are not a perfect measure of intruder pressure. They do not capture takeover attempts that leave no evidence and do not account for the fact that more aggressive females suffer fewer ovicide losses

(see Chapter 4, Figure 4.3). However, in combination with other evidence, it suggests birds perceive territories that are routinely settled earlier as high quality resources.

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CONCLUSION

Social competition shapes both male and female phenotypes in diverse and dynamic ways. Here I investigate the form and function of two understudied competitive traits once thought to be exclusive characteristics of males: female song and intrasexual aggression. My work on a Michigan population of house wrens (*Troglodytes aedon*) illustrates three broad themes there are useful for understanding female competitive traits in other species.

(1) Competitive traits in female house wrens are not merely byproducts of selection on males but instead play functional roles in female lives. Female song was common in this population (Chapter 1), despite the belief that temperate zone female song is generally rare. Females sang most often during the times that conspecifics were the biggest threat to female reproduction and females dramatically elevated singing rates during simulated conspecific intrusions (Chapter 1). Female vocalizations did not seem to be just random noise. Females that sang more in response to simulated conspecifics ultimately lost fewer eggs to real intruding house wrens, suggesting that female song is an important component of female house wren nest defense (Chapter 1). Similarly, listening females responded differently to different types of female vocalizations (Chapter 2). One particular vocalization, the HI call, functioned as an aggressive signal during female interactions (Chapter 2). Like female song, female intrasexual aggression had an important function for female house wrens. Females compete with one another for access to nest cavities (Chapter 4). Females that were more aggressive toward simulated female intruders were more likely to win contests over limited nest cavities and more successful at protecting their nests from intruding conspecifics during periods of elevated competition (Chapter 4). Furthermore, females flexibly increased their aggressive response

when they were at greater risk of losing the nesting cavity (Chapter 3). Female song and female intrasexual aggression have important reproductive consequences for female house wrens.

(2) While female competitive traits have some similarities to the same traits in males, they are best understood by explicitly considering the contexts in which females compete and the resources that affect female fitness. Male house wrens use song and aggression to compete with other males for access to mates and to defend territories. Likewise, females used song and aggression to defend nests (Chapter 1, Chapter 3, Chapter 4). However, focusing on patterns from males may lead us to miss important details of female competition. For instance, although males sing throughout the nest cycle, female song was only very common during the beginning of the egg laying period (Chapter 1). This is likely the result of sex-differences in competition. Males use these competitive traits to attract females and defend territory boundaries throughout the season. In contrast, females may compete to gain a nest cavity (Chapter 4), ward off potentially ovicidal intruders (Chapter 1, Chapter 4), and prevent a secondary female from settling (Chapter 1). All of these scenarios occur during narrower windows of time. Unlike males, females did not appear to aggressively compete for access to mates (Chapter 3, Chapter 5). Instead, the nesting cavity appeared to be most important to female competition (Chapter 3, Chapter 4). Furthermore, female aggressive competition appears to have benefits unique to females. More aggressive females, but not more aggressive males, protected a larger proportion of their eggs from house wren ovicide and raised larger offspring (Chapter 4, Chapter 5). Focusing only on the contexts and benefits of male competition is a poor approach to understanding female competitive traits.

(3) The same female trait can affect multiple components of female fitness. An organism's phenotype is the cumulative result of selection acting on multiple components of

fitness. However, researchers lack a comprehensive understanding of the costs and benefits to female competitive traits in most species. Researchers often infer costs to female competitive traits using predictions from theory or patterns from males. Here I show that female intrasexual aggression helped females win contests over nesting cavities (Chapter 4), protect eggs from house wren ovicide (Chapter 4), raise larger offspring (Chapter 5), and ultimately fledge a higher proportion of offspring (Chapter 5), although they may have been less likely to return the subsequent year (Chapter 5). These patterns contrast with patterns found with males in some species. Overall, this work emphasizes that understanding how competitive traits are shaped in both male *and* female bodies is critical to a broad understanding of the evolution of competitive traits.