Female song in the superb fairy-wren (*Malurus cyaneus*): perspectives informed by function and ontogeny

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Thesis Summary

Songbirds (oscine passerines), the largest Order of birds, have intrigued humans for centuries given their capacity to learn complex vocalisations and produce complex songs. Despite the fact that female songbirds also sing, the vast majority of research into song learning, as well as the functions of song, has come from studies on male birds. My thesis asks and answers some fundamental questions about whether there are differences in song learning in sons and daughters when both parents produce complex solo song, and if there are gender-specific differences in the costs of adult song. I use a model Australian songbird system with male and female song, the superb fairy-wren (*Malurus cyaneus*). Using observational and experimental cross-fostering approaches, my thesis shows that male and female fledglings produced songs of comparable complexity and sang song element types of (foster) social mothers and fathers. I conclude that both adult males and females were vocal tutors for young birds. Given that offspring learned the vocal elements of both parents, I tested if the male-centric hypothesis for mate choice by females explains pairing patterns whereby females with complex song should be paired with males with complex song. While this was rejected I instead found assortative pairing for element type (not complexity) whereby females with complex song were paired with males with many shared element types. Finally, there were gender differences in the costs of singing. Females that produced many chatter songs inside the nest had higher nest predation compared with males with high song rate close to the nest. Therefore, this thesis identifies singing as costly for females, but also shows that female singing behaviour plays a role to vocally tutor fledged young. I suggest that females may increase their fitness by selecting males with a shared vocal repertoire, to enhance the efficacy of territory defence. The outcomes of the thesis raise new theoretical

frameworks for sexual selection linked with shared territory defence (commonly referred to as social selection), as well as new ideas to test the function of female song for ontogenetic patterns when both sexes sing as adults and both sexes learn song during development.

Declaration

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.'

Christine Evans

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Statement of Authorship/Contribution

Chapters 1 & 6: CE

Chapter 2:
Data collection: CE
Statistical analyses: CE & SK
Manuscript writing: CE
Manuscript development and feedback: CE & SK

Chapter 3:
Data collection: CE & SK
Laboratory analysis of DNA samples: CE
Statistical analyses: CE & SK
Manuscript writing: CE
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Chapter 4:
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All research procedures reported in this thesis follow the guidelines for the use of animals in research (Flinders University), and were approved by the Animal Welfare Committee of Flinders University (permit numbers E234, E235, 236, E386). Permit to undertake scientific research in South Australia was granted by the South Australian Department of Environment, Water and Natural Resources (permit number Z24699). All birds were banded under permit (banding authority number 2601) from the Australian Bird and Bat Banding Scheme.

Publications associated with this thesis

Information from this thesis has been published in peer-reviewed journals as follows:

Evans, C., and Kleindorfer, S. (2016). Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers. *Frontiers in Ecology and Evolution*. 4: 9.

Kleindorfer, S., **Evans, C.,** and Mahr, K. (2016). Female in-nest chatter song increases predation. *Biology Letters*. 12: 20150513.

Related publications (included in appendix):

Kleindorfer, S., **Evans, C.,** Mihailova, M., Colombelli-Négrel, D., Hoi, H., Griggio, M., Mahr, K., and Robertson, J. (2013). When subspecies matter: resident Superb Fairy-wrens (*Malurus cyaneus*) distinguish the sex and subspecies of intruding birds. *Emu*. 113: 259-269.

Kleindorfer, S., **Evans, C.,** and Colombelli-Négrel, D. (2014). Females that experience threat are better teachers. *Biology Letters*. 10 (5).

Kleindorfer, S., Hoi, H., **Evans, C.,** Mahr, K., Robertson, J., Hauber, M, and Colombelli-Négrel, D. (2014). The cost of teaching embryos in superb fairy-wrens. *Behavioral Ecology*. 25 (5): 1131-1135.

Mahr, K., **Evans, C.,** Thonhauser, K. E., Griggio, M., & Hoi, H. (2016). Multiple ornaments—multiple signalling functions? The importance of song and UV plumage coloration in female superb fairy-wrens (*Malurus cyaneus*). *Frontiers in Ecology and Evolution*. 4 (43).

Chapter 1

General Introduction

Female song – an overlooked biological phenomenon

Bird song has been regarded as a sexually selected trait since Darwin (1871) proposed the idea. However, empirical evidence and experimental tests to support the seminal idea that (male) bird song could be a sexually selected trait (via female choice) only emerged in the second half of the twentieth century (Searcy and Yasukawa, 1996; Catchpole and Slater, 2008). It is now well established that male songbirds sing to repel rival males and attract and stimulate females (Catchpole and Slater, 2008). In order for a trait to confer its chooser with direct or indirect fitness, the trait must honestly signal quality (Andersson, 1994). This was a key missing link in understanding how song could be the target of sexual selection, as it was unclear how song signalled quality. Experimental studies have shown that a range of song characteristics honestly signal male quality, in part because song development is influenced by early rearing conditions (Nowicki et al., 2002; Spencer et al., 2003; Buchanan et al., 2003; reviewed in Gil and Gahr, 2002). Males that developed under good rearing conditions had a larger song repertoire and more complex song as adults (Spencer et al., 2003, 2005; Buchanan et a., 2004). Males with these song parameters are often more successful at holding territories and attracting females (Catchpole, 1980; Hiebert et al., 1989; Spencer et al., 2005). These findings provided compelling support for the idea that song in male songbirds is an example of a behavioural trait shaped by sexual selection. Over the past decade – and hence very recently in the history of thought regarding the occurrence and function of song -

female song has emerged as an understudied but phylogenetically widespread phenomenon (Odom et al., 2014). There is little theoretical framework to interpret its ontogeny or function, because cases of male choice for female mates based on song (a line of enquiry that would satisfy the theory of sexually selected traits shaped by mate choice) appear to be almost non-existent. The global prevalence of female song highlights the need to re-evaluate its biological significance. This thesis takes up the challenge of examining female song in relation to pair male song, and also asks if sons and daughters learn from their maternal vocal tutors.

Most ornithological research has been done in the Northern Hemisphere, where female song has long been regarded as a rare trait (Morton, 1996; Slater and Mann, 2004). When female song did occur, it was considered an accidental outcome of genetic correlations with males or due to hormonal imbalances (Lande, 1980; Amundsen, 2000; Byers and King, 2000; Catchpole and Slater, 2008). As research on songbirds expanded into tropical and southern temperate regions, female song was increasingly observed and found to be common in these regions (Robinson, 1949), yet often remained ignored in birdsong literature (Riebel, 2003; Riebel et al., 2005). The recent study of Odom and colleagues (2014) showed that female song was most likely present in the ancestor of songbirds, and females sing in 71% of extant species across 32 families. Consequently, common perceptions about song behaviour and the evolution of song in oscine passerines are currently being questioned in light of these findings. There is now a growing research focus on the occurrence and functions of female song as well as the underlying causal mechanisms and its development, with emerging examples from ancient lineages in the Southern Hemisphere.

Male and female song learning

In contrast to males, it was previously thought that females were incapable of learning and producing complex song. Vocal learning has been studied extensively in male songbirds, and has additionally been inspired because of the parallels between the development of birdsong and human speech. This vast body of work has shown that there are fundamental differences in the song control system of the brain in male and female songbirds, and that males generally learn and produce more complex songs than females (MacDougall-Shackleton and Ball, 1999; Catchpole and Slater, 2008). Males often have larger song nuclei volumes than females, and this is most apparent in systems with male-only song production, like the model species for the study of bird song: the zebra finch (*Taeniopygia guttata*; Nottebohm and Arnold, 1976; MacDougall-Shackleton and Ball, 1999; Garamszegi et al., 2005). Thus, this male-biased sexual dimorphism in the volume of the song control system of the brain was associated with the rarity of complex song in females (Nottebohm and Arnold, 1976; Arnold 1992). However, we now know that adult females can produce complex solo song, and can coordinate their songs to produce duets with their partner (Levin, 1996; Langmore, 1998; Riebel, 2003; Brunton and Li, 2006; Pilowsky and Rubenstein, 2013; Illes, 2015; Schwabl et al., 2015). In some species, male and female song can be similar in spite of male-biased sexual dimorphism of song nuclei volume (Lobato et al., 2015; Schwabl et al., 2015). Sex differences or similarities in song may also arise from the age at which song is learned. Furthermore, males and females may show sex-specific differences in song learning and vocal tutor preferences that contribute to sex differences in adult song production. This thesis addresses the research gap in song learning in species with male and female song production.

Females as vocal tutors

Young birds require exposure to singing birds that act as vocal tutors during a sensitive phase of song development, since song is at least partially acquired by imitation (Catchpole and Slater, 2008). Young male songbirds may copy and learn from adult males that they socially interact with, such as their fathers, or territorial neighbours (Böhner, 1983; Grant and Grant 1996; Roper and Zann, 2006). Often, males learn and imitate songs from several adult males to develop a larger song repertoire or more complex song. Previously, females were seldom recognised as having an active role in song learning since female song was considered rare. Subsequently, the role of females as vocal tutors in systems with male and female song production has been understudied, and it is unclear whether young birds learn from same-sex vocal tutors, opposite-sex vocal tutors, or both (Riebel, 2003, 2016). Research on captive Northern cardinals (*Cardinalis cardinalis*) and blue-capped cordon-bleus (*Uraeginthus cyanocephalus*) suggests that males and females are capable of learning from both sexes and produce similar songs as juveniles, despite sex differences in adult song (Yamaguchi, 2001; Geberzahn and Gahr, 2013). Emerging research shows that females are important vocal tutors for young fairy-wrens (Colombelli-Négrel et al., 2012; 2016; Kleindorfer et al., 2014a; Dowling et al., 2016), yet further work is required to understand sex differences in song development and vocal tutor preferences. I examine the role of females (and males) as vocal tutors for song acquisition in songs and daughters.

A new framework for testing functions of female song

The conceptual framework for understanding the evolution of female song remains a complex issue because different selection processes may govern female song in comparison to male song. In order to understand the selection processes acting on bird song in general, I firstly define natural, sexual and social selection. Natural selection is the differential survival and reproduction of individuals based on heritable phenotypic differences that arise from environmental selective pressures, and hence drive adaptive change across generations (Darwin, 1859; Grant, 1991). Sexual selection is a mode of natural selection that acts on secondary sexual traits that affect an individual's reproductive success, which arises from mate choice and intrasexual competition for access to mates (Darwin, 1871; Andersson, 1994). Social selection involves the selection of phenotypic traits associated with social competition to gain access to breeding and ecological resources (West-Eberhard, 1979; 1983). Male song is regarded as a classic example of a sexually selected trait because it is shaped by female choice and male-male competition (Searcy and Yasukawa, 1996; Gil and Gahr, 2002; Catchpole and Slater, 2008). In contrast, the function of female song as understood to date, has shown that it plays a role in female-female competition for resources important for fecundity (mates, nest sites) and survival (foraging territories) (Langmore, 1998; Cooney and Cockburn, 1995; Hall and Peters, 2008; Illes and Yunes-Jimenez, 2009; Cain et al., 2015; Dalziell and Welbergen, 2016). Female song rarely functions for mate attraction (but see Langmore et al., 1996), which has been explained by the fact that females are generally the 'choosy sex' because they have higher energetic investment into gametes and parental care than males (Andersson, 1994). For these reasons, sexual selection is a more unsatisfactory explanation for the evolution of female song than for male song. To overcome this limitation, social selection theory is often applied to female song because it is associated with social competition for both sexual and ecological resources (Odom et al., 2014). The haziness emerges because female song may also be under sexual selection when including intrasexual competition for breeding opportunities or resources that affect fecundity, rather than solely competition for mating opportunities (Clutton-Brock, 2007). We therefore must either extend the concept of sexual selection (discussed in Clutton-Brock, 2009) or apply the broader framework of social selection (West-Eberhard, 1979; 1983) to understand the evolution of female song, whereby the limitations of these theoretical frameworks remain unresolved (reviewed in Clutton-Brock, 2009; Tobias et al., 2012). To shed light on this issue, one can identify the fitness costs and benefits of female song to understand underlying evolutionary mechanisms of selection. For male songbirds, recognised fitness costs of singing include the high energetic costs of production (Eberhardt, 1994), and increased risk of predation (Møller 2006; Møller et al., 2008). Yet males clearly increase their survival and fecundity by singing. The costs of singing for females must be high since females generally sing less frequently than males, and produce shorter, less complex songs (Hall et al., 2015). Alternatively, the benefits of singing for females may be smaller than for males, which could also lead to sex differences in singing behaviour. This thesis explores gender differences in potential costs and benefits of song.

Pairing patterns of male and female song

Females show a preference for males that produce complex song or have a large element or song repertoire (Catchpole and Slater, 2008). There is high individual variation in these song characteristics, and they are honest signals of quality (Gil and Gahr, 2002; Nowicki et al., 2002; Buchanan et al., 2003; Spencer et al., 2003). Song production and complexity have also been associated with measures of fitness in female songbirds (Langmore et al., 1996; Pavlova et al., 2010; Cain et al., 2015;

Brunton et al., 2016). Therefore, female song may also reflect individual quality. As female song mainly functions for resource defence (Langmore, 1998), it suggests that song complexity in females is driven by female-female competition, rather than mate choice. However, it is yet to be investigated whether females that sing maintain a preference for males with complex song, or prefer males that produce similar songs. I examine assortative pairing for song complexity within paired males and females.

Malurus as a model system for understanding female song and vocal learning

Species in the *Malurus* genus are characterised by cooperative breeding, sexual dichromatism, and song production by males and females (Rowley and Russell, 1997). Fairy-wrens are a model system to study female song given their complex song structure and singing behaviour, and both sexes sing year-round (Rowley and Russell, 1997). The *Malurus* genus is one of few genera where male and female song has been described and the functions of song have been identified for multiple species, including the superb fairy-wren (*M. cyaneus*; Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Kleindorfer et al., 2013b; Cain and Langmore, 2015; Cain et al., 2015), splendid fairy-wren (*M. splendens*; Greig and Pruett-Jones, 2008, 2009; Colombelli-Négrel, 2016), variegated fairy-wren (*M. lamberti*; Colombelli-Négrel, 2016), purple-crowned fairy-wren (*M. coronatus*; Hall and Peters, 2008), and red-backed fairy-wren (*M. melanocephalus*; Dowling and Webster, 2013; Schwabl et al., 2015; Dowling et al., 2016; Baldassare et al., 2016). Therefore, a comparative approach can be taken to examine patterns of singing behaviour and vocal learning. Male and female fairy-wren songs (solo song and duets) are mainly used for territorial defence and during dawn chorus displays (Langmore and Mulder, 1992; Rowley and Russell, 1997; Dalziell and Cockburn, 2008; Greig and Pruett-Jones, 2008, 2009; Hall and Peters, 2008; Dowling and Webster, 2013; Baldassare et al., 2016; Colombelli-Négrel, 2016). Recent research on vocal learning and production in *Malurus* species showed that sex-specific song learning occurs in splendid fairy-wrens (Greig et al., 2012), and red-backed fairy-wren adult offspring produce the song elements acquired from their mother (Dowling et al., 2016). Superb and red-backed fairy-wren females vocally tutor their young because they produce a specific call to eggs and nestlings that young birds learn and produce in their begging call (Colombelli-Négrel et al., 2012; Colombelli-Négrel et al., 2016; Dowling et al., 2016). Together, this research highlights the importance of fairy-wrens as a model system for male and female vocal learning and song production.

The superb fairy-wren is one of few species where female song has been investigated in detail, and is the best-studied fairy-wren and Australian bird in general. Like all fairy-wrens, it is a highly vocal species and there are eight described superb fairy-wren vocalisations (Rowley, 1965; Kleindorfer et al., 2013b). I study chatter song (Type I song) because it is the most common song produced by males and female year-round for resource defence (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Kleindorfer et al., 2013b; Cain and Langmore, 2015). Female chatter song and singing behaviour have recently been associated with body condition (Mahr et al., 2016) and reproductive success (Cain et al., 2015), suggesting that female song reflects individual quality. However, it is not known how chatter song develops in males and females in this species, or how female song attributes predict mate choice for a male partner that also sings. Furthermore, it remains untested whether there are sex differences in singing behaviour and how these differences reflect predation costs of singing behaviour. To address these questions, we monitored wild populations of superb fairy-wrens in South Australia over three years. Superb fairy-wrens live in a complex and variable social environment, which would be difficult to replicate in captivity. Despite the necessity to study animals in the wild as many cannot be realistically or ethically held in captivity, the study of wild populations can be very challenging and has many limitations. There are many variables that influence behaviour that cannot be measured or controlled for, in addition to the high predation of young and adults, the challenge to make quality recordings of identifiable individuals, and the influence of neighbours on individual behaviour. Nonetheless, field studies are vital for providing insights into behaviour and survival, and to improve our understanding of complex social and acoustic environments in other species. My thesis provides insights into how young fairy-wrens develop their song and the costs and benefits singing adult fairy-wrens face in their natural environment.

Thesis scope and objectives

This study investigates song development of male and female superb fairy-wren fledglings, and fitness consequences of adult male and female song behaviour. Specifically, this thesis aims to (1) examine whether young males and female produce song element types of both sexes, (2) uncover how element types are acquired by fledglings, (3) explore patterns of song complexity in social pairs, and (4) identify gender-specific differences in costs of adult singing behaviour.

Organisation of this thesis

This thesis consists of a series of manuscripts that are published, or in preparation for publication in peer-reviewed scientific journals. There is some repetition of content because each chapter is presented as a separate manuscript. The chapters are presented in the following order:

1. General Introduction

2. Evans, C., and Kleindorfer, S. (2016). Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song element types of social mothers and fathers. *Frontiers in Ecology and Evolution*. 4: 9.

3. Evans, C., and Kleindorfer, S. (in preparation). Cross-fostering shows that superb fairy-wren fledglings acquire song elements through social transmission. *Behavioral Ecology*.

4. Evans, C., and Kleindorfer, S. (in preparation). Solo female song within pairs: assortative pairing for element type. *Animal Behaviour*.

5. Kleindorfer, S., Evans, C., and Mahr, K. (2016). Female in-nest chatter song increases predation. *Biology Letters*. 12: 20150513.

6. Discussion

Chapter 2

Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers

Christine Evans and Sonia Kleindorfer

Frontiers in Ecology and Evolution (2016) *4*(9): 181-190

Abstract

Birdsong is regarded as a classic example of a sexually selected trait and has been primarily studied in systems with male song. Complex solo female song is emerging from the shadows of overlooked phenomena. In males, rearing conditions affect male song complexity, and males with complex songs are often more successful at mate attraction and territorial defence. Little is known about the ontogeny or function of complex female song. Here, we examine song elements in fledgling superb fairy-wrens (*Malurus cyaneus*) in relation to the song elements of adult tutors. Male and female superb fairy-wrens produce solo song year-round to defend a territory. We ask if sons and daughters acquire song elements from sex-specific vocal tutors. We found that sons and daughters produced the song elements of their mothers and social fathers, and that sons and daughters had comparable song element repertoires at age 7-10 weeks. We conclude that sons and daughters increase their song element repertoire when vocally imitating elements from several vocal tutors, and that both sexes acquire elements from male and female vocal tutors in this system.

Introduction

Birdsong is widely regarded as a sexually selected trait given ample evidence that complex male song is more likely to attract females and repel intruder males (Andersson, 1994; Marler and Slabbekoorn, 2004; Catchpole and Slater, 2008). There is growing evidence that female song is ‘common’ rather than ‘exceptional’ (Riebel et al., 2005; Garamszegi et al., 2007; Price, 2009; Odom et al., 2014), which focuses research attention on the role of sexual selection for complex female song (Price, 2015). Song complexity in females varies greatly across species (Odom et al., 2014; Price, 2015). As discussed by Price (2015): “Although female song is often treated as a discrete binary character in comparisons among species, with some species categorized as having female song and others not (e.g., Garamszegi et al., 2007; Price, 2009; Odom et al., 2014), evidence suggests that female singing can vary continuously across taxa in both expression and complexity.” To date, we know very little about how this complexity in female song arises.

Female song functions for multiple purposes, including territorial defence, mate attraction, coordination of breeding activities, and female-female competition for reproductive resources (Langmore, 1998). In the superb fairy-wren (*Malurus cyaneus*), female song, similar to male song, has been shown to function in response to the threat of same-sex conspecific intruders in the territory (Kleindorfer et al., 2013b; Cain and Langmore 2015). In banded wrens (*Thryophilus pleurostictus*), there is support for the function of female song for communicating with their breeding partner (Hall et al., 2015), whereas the main functions of male song are mate attraction and stimulation, and territorial advertisement and defence (Kroodsma, 1976; Catchpole and Slater, 2008). Therefore, the evolution of male and female song could be under the same or different selection pressures depending on the specific context (Price, 2015).

Song can be a sexually dimorphic trait. Generally, males sing more complex songs than females (discussed in Brenowitz and Kroodsma, 1996; Catchpole and Slater, 2008). Some studies have found comparable song structure, complexity, and repertoire size in males and females (Brunton and Li. 2006; Pilowsky and Rubenstein, 2013; Schwabl et al. 2015). While females rarely have larger song repertoires than males, there are exceptions: female stripe-headed sparrows (*Peucaea r. ruficauda*) have been shown to have more complex chatter song repertoires than males (Illes, 2015), and female Australian magpies (*Gymnorhina tibicen*) had larger duet repertoires than males (Brown and Farabaugh, 1991). The capacity to learn complex songs is strongly influenced by the development of the neural song system (Buchanan et al., 2004). In general, male songbirds have larger high vocal centre (HVC) nuclei than females (MacDougall-Shackleton and Ball, 1999; Hall et al., 2010), yet female song can be similar or more complex than male song (Illes, 2015; Schwabl et al., 2015). This suggests that a smaller HVC in female songbirds is not necessarily a constraint for learning and developing complex songs.

There is limited knowledge of song learning in females, including how females learn to sing and from whom (Riebel, 2003; Riebel et al., 2005). In species with male and female song, whereby the song may be the same or different across the sexes, both sexes are potential song tutors. When both parents sing, young birds could learn their song from both parents or from same-sex vocal tutors. Studies on captive birds show that young male and female songbirds learn primarily from same-sex vocal tutors in slate-coloured boubous (*Laniarius funebris*; Wickler and Sonnenschein, 1989), stripe-backed wrens (*Campylorhynchus nuchalis*; Price, 1998), and European starlings (*Sturnus vulgaris*; Hausberger et al. 1995). Young birds learn from both male and female song tutors in Indian Hill mynahs (*Gracula religiosa*; Bertram, 1970), Northern cardinals (*Cardinalis cardinalis*; Yamaguchi, 2001) and blue-capped cordon-bleus (*Uraeginthus cyanocephalus*; Geberzahn and Gahr, 2013; Lobato et al., 2015).

Our study species is the superb fairy-wren, a long-lived (up to 11 years) sedentary and territorial songbird (Rowley and Russell, 1997; Dunn and Cockburn, 1999). Both males and females sing solo chatter song year-round (Cooney and Cockburn, 1995; Cain and Langmore, 2015). The chatter song has several proposed functions including territory defence, mate attraction and within-pair communication (Cooney and Cockburn, 1995; Cockburn et al., 2009; Cain and Langmore, 2015). Recent evidence suggests that the primary function of chatter song is intrasexual competition for resources including social mates and territories (Cooney and Cockburn, 1995; Cockburn et al., 2009; Kleindorfer et al., 2013b; Cain and Langmore, 2015; Cain et al., 2015). A secondary function of chatter song may be within-pair communication because females sing on the nest in response to their social male’s song (Kleindorfer et al., 2016). It remains untested if males and females pair assortatively for song.

We study song element types in the subsong of fledgling superb fairy-wrens in relation to the song element types of the social father and mother, and ask if there are sex-specific vocal tutors. Individual fairy-wrens have different element types per song (Kleindorfer et al. 2013b); therefore, we predict within-pair differences in chatter song element types. Within pairs, we predict that the male and female will have ‘shared’ element types produced by both members of the pair and ‘within-pair unique' (hereafter 'unique') element types produced by only the male or female within the pair (but not necessarily unique to the population). We predict that sons and daughters sing the 'shared' parent element types because irrespective of vocal tutor type (male or female), the 'shared' element types will be present in the vocal repertoire of the tutor(s). We also predict that sons and daughters produce different proportions of 'unique' elements, whereby sons produce the 'unique' element in the social father's element repertoire and daughters produce the 'unique' element in the mother's element repertoire. This prediction rests on the idea that sons and daughters learn element types that signal their gender because birds acoustically discriminate sexes and chatter song functions for intrasexual competition (Cockburn et al., 2009; Kleindorfer et al., 2013b; Cain and Langmore, 2015). Finally, we predict that the presence of helper males in natal groups will affect element repertoire size in male and female offspring. If there is sex-specific vocal tutoring, then we predict that the presence of helper males will increase element repertoire size in the subsong of sons but not daughters. If, however, the number of vocal tutors (and not their gender *per se*) increases element repertoire size in both sons and daughters, then we predict that the presence of helper males in natal groups increases element repertoire size in the subsong of sons and daughters.

Materials and Methods

Study sites

This study on song element acquisition in the superb fairy-wren was carried out at two field sites (1) Cleland Wildlife Sanctuary (34°58'S, 138°41'E) and (2) Scott Creek Conservation Park (35°05'S, 138°41'E) in the Mount Lofty Ranges, South

Australia. We recorded adult chatter song and fledgling subsong from 11 family groups across three field seasons (September – February 2012, 2013, 2014).

Study species

The superb fairy-wren is an insectivorous passerine found in south-eastern Australia, and is a member of the Maluridae family (Rowley and Russell, 1997). The superb fairy-wren has a cooperative breeding system with a socially monogamous male and female pair, often assisted by one or more subordinate males (helpers) that provision the young and defend the permanent territory (Rowley 1965; Mulder et al., 1994; Dunn et al., 1995; Rowley and Russell, 1997; Mulder, 1997; Cockburn et al., 2008). The dominant male is not always the genetic father because most broods (75-95%) contain young sired by extra-pair males (Mulder et al., 1994; Cockburn et al., 2003; Colombelli-Négrel et al., 2009). We refer to the dominant male as the social father. The breeding season occurs between August and February, with 1-3 breeding attempts per year and 2-3 eggs per nest (Colombelli-Négrel and Kleindorfer, 2009). The incubation phase has a duration of 12-15 days and the nestling phase is 10-15 days (Colombelli-Négrel and Kleindorfer, 2009). Nest predation is high (e.g. 24-74%) (discussed in Rowley and Russell, 1997; Colombelli-Négrel and Kleindorfer, 2009) and once fledged, there is the risk of fledgling predation (Rowley, 1965; Cockburn et al., 2008). Most groups produce one brood of fledglings per year (Rowley and Russell, 1997). Females are uniparental incubators; all group members feed nestlings and dependent fledglings (Mulder et al., 1994; Dunn et al., 1995). Fledglings become independent of adult feeding around four weeks after fledging, but remain in the natal group for several months (Mulder, 1995; Rowley and Russell, 1997). Males are philopatric, remaining in the natal territory for one or more years as helper males, whereas females disperse in the first year, on average 1-10 km and 11.8 territories removed from the natal territory (Rowley, 1965; Cooney and Cockburn, 1995; Mulder, 1995; Cockburn et al., 2003; Double et al., 2005). Adult males and females are sexually dichromatic and can be easily distinguished in the field. Recently fledged young of both sexes resemble adult females (Mulder, 1995).

There are eight vocalizations described for the superb fairy-wren (Rowley, 1965; Kleindorfer et al., 2013b). We focus on chatter song (Type I song) because it is the most common song produced year-round by both male and female superb fairy-wrens for territorial and resource defence (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Kleindorfer et al., 2013b; Cain and Langmore, 2015). The chatter song is a variable, complex song that consists of approximately eight structurally distinct element types produced approximately 50 times per song for a duration of approximately 3 s (Langmore and Mulder, 1992; Kleindorfer et al., 2013b). Fledglings begin singing subsong from four weeks after fledging (Rowley, 1965; Langmore and Mulder, 1992). It is not known when fledgling subsong crystallises, but one-year old birds sing full adult song (Rowley, 1965). Adult males sing longer, more complex songs than females in some populations (Kleindorfer et al., 2013b). Males also have a larger song repertoire than females: males sing chatter song and trill song (Type II song) to attract extra-pair copulations (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Dalziell and Cockburn, 2008; Cockburn et al., 2009; Colombelli-Négrel et al., 2011). There is evidence that male trill song is learned: introductory elements of trill song were more similar between males and their social fathers than males and their genetic fathers; males that dispersed from the natal territory acquired the local trill song dialect (Blackmore, 2002). Furthermore, sons have been shown to learn Type II song (similar to superb fairy-wren trill song) from their social fathers in the splendid fairy-wren (*M. splendens*), a closely related *Malurus* species (Greig et al., 2012).

Nest monitoring

We monitored a total of 125 superb fairy-wren nests over three years. Nests were monitored every 2-4 days to check the status of the nest (building, eggs, nestlings, fledged) and nesting outcome (eggs depredated, eggs abandoned, nestlings depredated, nestlings abandoned, nestlings fledged). Of these 125 nests, 82 were depredated (65%) and 19 nests produced fledglings (15%). In this study, we analysed song recordings for 11 nests (49 birds) for which we have recordings of both parental chatter song (male and female) (N = 22 birds), helper males (N = 9) as well as all offspring (N = 17). For each nest, we had a minimum of three song recordings per individual bird (dominant male, dominant female, helper male, fledged young).

Of the 31 adult birds for which we have song recordings, we colour banded and measured 18 birds using target mist netting. We banded at least one dominant male or female at 10 nests and six helper males at the five nests with helper males. For individuals that were not banded, we were certain of their identity based on group size and composition and the interactions of unbanded birds with banded birds (e.g. group foraging together, feeding fledglings). Nine out of 11 nests contained a single unbanded bird; one nest had an unbanded dominant male and female; one nest had an unbanded dominant female and helper male. Each captured individual was marked with a unique combination of plastic colour bands and a numbered aluminium band provided by the Australian Bird and Bat Banding Scheme (ABBBS). Nestlings were banded 7-8 days after hatching.

We sexed the fledglings using the standard avian sexing method outlined by Griffiths et al. (1998), using primers P8 (5'-CTC- CCAAGGATGAGRAAYTG-3') and P2 (5'-TCTGCATC- GCTAAATCCTTT-3') with modifications to the protocol as follows. We carried out PCR amplification in a total volume of 24 µl with PCR reagents in following final concentrations: 1 X µM MRT buffer, 0.2 µM of each primer, 0.5 units Immolase and between 10-100 ng DNA. PCR conditions were an initial denaturing step at 94°C for 10 min, followed by 35 cycles of 94°C for 45 s, 48°C for 45 s and 72°C for 45 s. The program was completed with a final run of 72°C for 5 min and 25°C for 2 min. Of the 17 fledglings, eight were male and nine were female.

The research was approved by the Animal Welfare Committee of Flinders University (permit number E386), which operates under the *Animal Welfare Act 1985* (SA). Permit to undertake scientific research in SA was granted by the SA Department of Environment, Water and Natural Resources (permit number Z24699-11). All birds were banded under permit (banding authority number 2601) from the Australian Bird and Bat Banding Scheme.

Song recordings

We recorded the chatter song of 11 dominant pair males, 11 dominant pair females, nine helper males, eight sons, and nine daughters, from 11 nests. We recorded adult birds opportunistically throughout the breeding season. We recorded the song of fledgling birds at a distance of 5-10 m from the bird every 14 days for eight weeks post-fledging. Recordings were made with a Telinga Twin Science parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Device 722 digital audio recorder (Sound Devices, USA). Sound files were recorded as broadcast wave files (24 bit, 48 kHz).

Acoustical analyses

We transcribed all sound files to a Mac Pro (Apple Inc, USA) for editing with Amadeus Pro 2.1.2 (HairerSoft, Switzerland) and analysis with Raven Pro 1.5 (Charif et al., 2010). Spectrograms were created for 3-5 songs per individual using the Hann algorithm (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DTF 256 samples). We scored the number of different element types per song for every bird. We refer to the number of different element types per bird, summed over the 3-5 songs analysed, as the song element repertoire. In this study, we did not quantify the absolute element repertoire size. Using the available data of 3-5 songs per individual, there was no statistical association between the number of songs analysed per bird and the element repertoire size. We refer to the combined number of different element types between two or more individuals (for example, the pair) as song element diversity. We define an element in the song as a single, continuous trace on a spectrogram. We created an element library (Figure 2.1) based on the existing element classifications developed by Langmore and Mulder (1992), Blackmore (2002), Dalziell and Cockburn (2008), Colombelli-Négrel et al. (2011), and Kleindorfer et al. (2013), and comparing these classifications of elements to songs of our monitored populations. We identified 10 element types that have previously been classified in different populations (A, F, O, P, Q, R, T, U, V, W,) and 6 new elements found in our populations (FL, G, K, L, Z, ZN). Within pairs, we identified element types that were present in both the male and the female songs; these element types are referred to as ‘shared’ elements. Within pairs, we identified element types that were present in either the male or the female; these element types are referred to as ‘within-pair unique elements’ (hereafter referred to as ‘unique’; we note that within-pair ‘unique’ elements may occur in other fairy-wren individuals and hence are not ‘unique’ to the individual but just within the pair).

We used spectrogram cross-correlation (SPCC) analysis to examine the similarity between different element types, using five examples from different individuals per element type (Raven Pro 1.5, Cornell Lab of Ornithology; band-pass filtered from 500 Hz to 12000 Hz). SPCC produces a matrix of similarity (S), which we transformed into a matrix of distance (D) using the transformation [D = (1 – S) 0.5]. This matrix was evaluated by principal coordination analysis (PCoA) using the R package for multivariate and spatial analysis, version 4.0 (Casgrain and Legendre, 2001), as outlined in Colombelli-Négrel et al. (2012). The PCoA gives several coordinate values per element and the first five coordinate values explain most of the data set, which we used as similarity values (Baker and Logue, 2003). To determine if the assigned element categories were significantly different from one another, we averaged the five PCoA coordinate values into a single measure and analysed the mean coordinate values per element type in a univariate ANOVA using IBM$^{\circledR}$ SPSS$^{\circledR}$ 22 (SPSS Inc., Chicago, USA). The 16 different element types were significantly different ($F_{14, 60} = 1.919$, $P = 0.042$). We used these element categories to classify the different element types per song, and the song element repertoire per individual (Figure 2.1). We printed spectrograms and assigned element types visually according to the library of element categories. This method was chosen because humans outperform machines for tasks like the visual recognition of element types (Law and von Ahn, 2011). Three people (two naïve assessors and the person who scored the spectrograms for this study) independently reviewed 20 randomly chosen spectrograms (identity of bird unknown) and classified the different element types. The average similarity rate was 95.7% for the scoring of the number of different element types.

Statistical analyses

We used IBM® SPSS® 22 for statistical analyses (SPSS Inc., Chicago, USA) for statistical analyses of song element types. We examined the data for normality and homogeneity of variance. The variables ‘different element types per social father’ and ‘different element types per mother’ were square root transformed, and ‘number of unique paternal elements’ and ‘number of unique maternal elements’ were reflect and square root transformed to satisfy requirements of normality for parametric tests.

Results

Song element repertoire of social father and mother pairs

There were 16 different song element types in the repertoire of the 11 males and 11 females that were social parents (Figure 2.1). No element was exclusive to males or females in the study population, and hence there were no ‘male elements’ versus ‘female elements’. Element repertoire size was statistically comparable between social fathers (8.5 ± 0.6; mean ± se) and mothers (8.5 ± 0.4) for 11 pairs (paired samples t-test: $t_{10} = 0.064$, $P = 0.950$). The element diversity (combined element repertoire of social father and mother) between pairs varied (mean: 10.7 ± 0.4, range: 9 - 14).

Within each pair, 60.2 ± 3.7% of song elements were present in both the social father and mother song, and these elements are referred to as ‘shared’. The percentage of elements present in only the social father or mother song was 39.8 ± 3.7%, and these elements are referred to as ‘unique’. The mean number of ‘shared’ element types within pairs was 6.4 ± 0.3 element types. The number of ‘unique’ elements did not differ significantly between social fathers (2.2 ± 0.6) and mothers (2.2 ± 0.3) (paired samples t-test: $t_{11} = 0.530$, $P = 0.608$) (Figure 2.2).

Song element repertoire of the sons and daughters

Song element repertoires were comparable in sons (9.3 ± 0.8) and daughters (10.2 ± 0.6) (independent samples t-test per nest: $t_{12} = -0.813$, N = 14 birds, P = 0.432) (Figure 2.2). The average number of different element types per brood varied (mean: 8.9 ± 0.5, range: 6 - 11.5). There was no significant correlation between the element diversity of the social father and mother, and brood (linear regression: $F_{1,9} = 0.119$, r = 0.114, P = 0.738) or group (parents + helper male) and brood (linear regression: $F_{1,9} = 0.028$, r = 0.955, P = 0.871).

‘Shared’ and ‘unique’ song elements present in sons and daughters

The number of ‘shared’ elements from their social parent vocal tutors was comparable in sons (5.6 ± 0.4) and daughters (6.5 ± 0.2) (sign test: $P > 0.999$) (Figure 2.3). Of the total song elements produced by fledglings, there was no significant difference between the percentage of ‘unique’ maternal elements (86.0 ±

10.3%) and the percentage of ‘unique’ paternal elements (64.3 ± 11.2%) (sign test: $P = 0.125$). Fledglings that sang ‘unique’ element types from both the mother and social father produced a higher number of different element types (10.3 ± 0.6) than fledglings that did not sing ‘unique’ element types from both social parents (7.3 ± 0.9) (independent samples t-test: $F_{10} = 0.417$, $t = -2.915$, $P = 0.015$). Looking at each sex separately (using the average per sex per nest), sons did not differ significantly in the number of ‘unique’ maternal (1.9 ± 0.2) or paternal (1.3 ± 0.5) elements (sign test: $P = 0.289$), and daughters did not differ significantly in the number of ‘unique’ maternal (1.2 ± 0.3) or paternal (1.5 ± 0.3) elements (sign test: $P > 0.999$) (Figure 2.3). Five fledglings had ‘unique’ element types (1.4 ± 0.2) not present in the song repertoire of the social father, mother or helper male.

Song element repertoire of helper male in relation to dominant pair male and female

Of the 11 nests, five nests had helper males (one nest had one helper male and four nests had two helper males). Among helper males, most element types (56.4% ± 2.8) were ‘shared’ with the dominant pair, some elements were the same as the ‘unique’ maternal element types (15.3% ± 3.9), some elements were the same as the ‘unique’ paternal element types (13.6% ± 3.9), and some were ‘unique’ to the helper male (14.6% ± 3.0). Expressed as a number rather than a percentage, helper males had 1.6 ± 0.4 ‘unique’ element types that were different from the ‘unique’ element types in the pair male and female. The number of different element types produced by all adult birds (pair + helper males) was not significantly different between nests with and without helper males (independent samples t-test: $t_9 = -1.393$, $P = 0.197$).

Song element repertoire of sons and daughters in relation to groups with and without helper males

There was no significant difference between the element repertoire size of fledglings in groups with helper males (9.6 ± 1.0) and without helper males (9.1 ± 0.7) (independent t-test: $t_{10} = -0.372$, $P = 0.718$). Examining each sex separately, the element repertoire size of sons in groups with helper males was comparable with the element repertoire size of sons in groups without helper males (independent t-test: $t_6 = -0.551$, $P = 0.601$); the element repertoire size of daughters in groups with helper males was comparable with the element repertoire size of daughters in groups without helper males (Mann-Whitney U test: $U = 4.000$, $z = -0.221$, $P = 0.825$) (Figure 2.4).

Discussion

The main findings of this study are that (1) the chatter song in nesting pairs of superb fairy-wrens contained ‘shared’ element types found in both male and female chatter song as well as ‘unique’ element types produced by only one parent (male or female); (2) sons and daughters sang ‘shared’ and ‘unique’ song element types of both social father and mother (parental) vocal tutors; (3) sons and daughters had comparable song element repertoires at age 7-10 weeks; (4) the presence of helper males did not increase the element repertoire size of fledglings. These findings suggest that sons and daughters acquire song element types from both male and female tutors. Because we did not assess maximum repertoire size in adult or fledgling birds or examine song element types of neighbouring birds, sons and daughters may produce vocal elements from individuals outside their social group.

Within-pair 'shared' and 'unique' song element types

Males and females within-pairs produced element types that were either present in the chatter song of both parents, or element types present in only one parent. Repertoire composition was variable across individuals, with no consistent sex differences in the presence or absence of particular element types. Other studies have found both sex-specific and individual-specific element types. Male and female bellbirds (*Anthornis melanura*) shared 20% of syllable types but most syllables (80%) were sex-specific at a population level (Brunton and Li, 2006). In comparison, male and female Australian magpies rarely shared syllable types for carol songs, and syllable repertoires were highly individual-specific (Brown and Farabaugh, 1991). Our study was limited to the comparison of shared and unique elements within pairs and not at the population level. At the population level, there were sex differences in the number of particular element types; similar to our study, there were no sex-specific element types (Kleindorfer et al., 2013b).

Sons and daughters sang element types of mothers and social fathers

Our finding that offspring produced the 'unique' maternal and 'unique' paternal element types supports the idea that superb fairy-wren mothers and social fathers were vocal tutors for sons and daughters. However, we did not compare the parental element repertoire with neighbouring individuals, so fledglings could also have learned these element types from individuals other than the social parents. Because we did not cross-foster clutches, we cannot rule out innate components of element repertoire emergence in sons and daughters. It is well-established that oscine passerines learn their songs (Brenowitz, 1991; Brainard and Doupe, 2002; Pfenning et al., 2014), and previous research has shown vocal imitation in fairy-wrens (Greig et al., 2012; Colombelli-Négrel et al., 2012). Therefore, it is reasonable to conclude that superb fairy-wren fledgling song elements were learned. The finding that sons and daughters produced ‘unique’ elements from mothers and fathers is an exciting finding because few studies to date have tested if both sexes may be vocal tutors for young male and female birds. Yamaguchi (2001) showed that captive Northern cardinals learn multiple song types from same-sex and opposite-sex vocal tutors, and Geberzahn and Gahr (2013) found that male and female blue-capped cordon-bleus also learn song from either sex, irrespective of the fact that males have larger syllable-type song repertoires than females. This is in contrast to other studies, which found that young birds showed a preference for learning vocalisations from same-sex vocal tutors (Wickler and Sonnenschein, 1989; Hausberger et al., 1995; Price, 1998).

Learning from two vocal tutors may increase an individual’s chances of acquiring complex song. A wealth of research has shown that song complexity is an honest signal of male quality (Buchanan et al., 2004; Spencer et al., 2005; Schmidt et al., 2013), important for female mate choice (Catchpole, 1980; reviewed in Byers and Kroodsma, 2009). In superb fairy-wrens, different male song types predicted extra-pair fertilisations (Dalziell and Cockburn, 2008), and sexes had different element complexity (Kleindorfer et al., 2013b). A theoretical framework for functions of female song complexity mostly focuses on resource defence (Cain and Langmore, 2015; Illes, 2015). Evidence for increased female fitness associated with complex female song comes from a few studies: older female alpine accentors (*Prunella collaris*) that sang more complex songs had larger clutch sizes (Langmore et al., 1996), and female European starlings with complex song had repeatable reproductive performance across years (Pavlova et al., 2010). Here, we show that offspring had larger song element repertoires when exposed to element types of different vocal tutors, but we did not examine fledgling song complexity. We expect that fledglings with greater song complexity will have greater fitness.

The cooperative breeding system of the superb fairy-wren means that young birds interact with all group members (Mulder, 1995; Rowley and Russell, 1997). Social interactions with vocal tutors have been shown to facilitate song learning (Baptista and Petrinovich, 1984, 1986; Beecher et al., 1994; Beecher and Burt, 2004). Research on zebra finches (*Taeniopygia guttata*) found that social interactions influenced song tutor choice: young males preferred male vocal tutors that provided a greater amount of parental care (Williams, 1990), and chose fathers over unrelated males (Eales, 1987). The role of social interactions for the attention of young birds for particular adult vocal tutors remains to be tested in our system. All adults provisioned all offspring, and fledglings produced 'shared' and 'unique' parental element types. Although groups with helper males had on average one more 'unique' element type per group, we did not find that fledglings of groups with helper males had a larger element repertoire than fledglings of groups without helper males. Helper males tend to be sons from a previous brood, and therefore these males also (largely) acquire their elements from the mother and social father. Perhaps the within-family element diversity is maintained rather than enhanced in the presence of helper males who may act as additional tutors.

Song element repertoire in sons and daughters

Previous research showed higher song complexity in adult males than females (Kleindorfer et al., 2013b). It is possible that daughters lose element types with age, as we did not find sex differences in fledgling element repertoire size. Such a decrease in repertoire size with age has been found in female blue-capped cordon-bleus (Lobato et al., 2015). Males may have a longer sensory acquisition phase than females and acquire more element types over a longer timeframe, which is another explanation for why adult males have more complex song than females. Such a process has been proposed for Northern cardinals and blue-capped cordon-bleus (Yamaguchi, 2001; Geberzahn and Gahr, 2013; Lobato et al., 2015). All we can say at present is that we found no sex differences in fledgling song element repertoire, so therefore it is likely that sex differences emerge later in the superb fairy-wren.

Although sons and daughters sang element types of both parents, pairs with higher element diversity did not always have offspring with higher element diversity. This suggests that the development of complex song could be influenced by factors other than the element diversity of parents (Buchanan et al., 2003, 2004; Spencer et al., 2005; Schmidt et al., 2013). Rearing conditions can influence song learning and development. There is ample evidence for negative effects of developmental stress on song learning and complexity in male songbirds. Research into zebra finches found that developmentally stressed males had smaller HVC nuclei (area of brain associated with production of complex songs), and therefore lower song complexity (Buchanan et al., 2004; Spencer et al., 2005). Similarly, swamp sparrows (*Melospiza georgiana*) that received less food early in life had poor copy accuracy and a smaller repertoire size, suggesting that malnutrition affects song learning (Nowicki et al., 2002). Therefore, the observed variation in the average number of different elements across broods in this study could be explained by nutritional or environmental stress

(Nowicki et al., 2002; Buchanan et al., 2004; Spencer et al., 2005), which we did not measure.

Exposure to song during development may have contributed to differences in the number of elements between broods. Offspring were exposed to both adult male and female song (Chapter 3). However, we did not test for differences in song rate between adult males and females in the presence of fledged young in this study. Variation in song rates and subsequent exposure to song may influence song learning and copy accuracy (but see Hultsch et al., 1999). In a separate study of incubation calling in superb fairy-wrens, Kleindorfer et al. (2014) found that increased call rates resulted in higher vocal copy accuracy of chicks. Perhaps some of the variation in whether chatter song elements were produced by young birds is explained by the song rate of the parental birds (purported vocal tutors).

Conclusion

There is much interest in the function of female song, its complexity, and whether it is also subject to sexual selection. In this study we focused on the acquisition of song elements in sons and daughters in a system where both sexes produce solo song year round as adults. Sons and daughters produced vocal elements that included the same proportion of ‘shared’ and ‘unique’ elements between the pair male and female attending the nest. Given that male and female song elements were present in the song of the sons and daughters, we conclude that the diversity of element types in the combined ‘parental repertoire’ was copied and produced by the entire brood, resulting in a ‘family vocal repertoire’. This finding raises several new lines of inquiry into song patterns within families. We conclude with two exciting questions here: (1) Do social partners have assortative pairing for element diversity (‘shared’ and ‘unique’) to increase song complexity in their offspring, and do offspring with higher vocal complexity have greater fitness? (2) Do social partners have disassortative pairing for ‘shared’ elements to reduce inbreeding?

Acknowledgements

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Figures

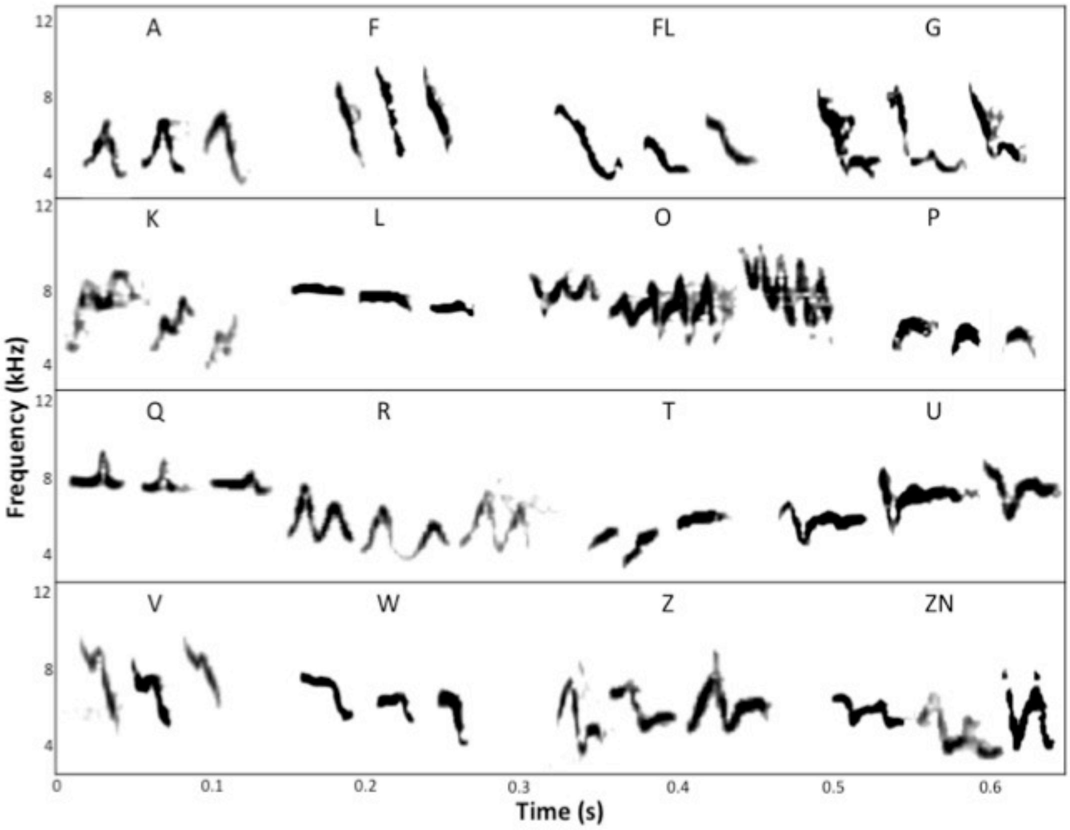

Figure 2.1 Spectrograms of different element types identified in the chatter song of superb fairy-wrens (*Malurus cyaneus*) from populations in the Mount Lofty Ranges region of South Australia. Each element type is illustrated with three exemplars. The elements identified in these populations include both existing element classifications and novel element types. The existing element types are: A, F, O, P, Q, R, T, U, V, W. The novel element types found in the studied populations are: FL, G, K, L, Z, ZN.

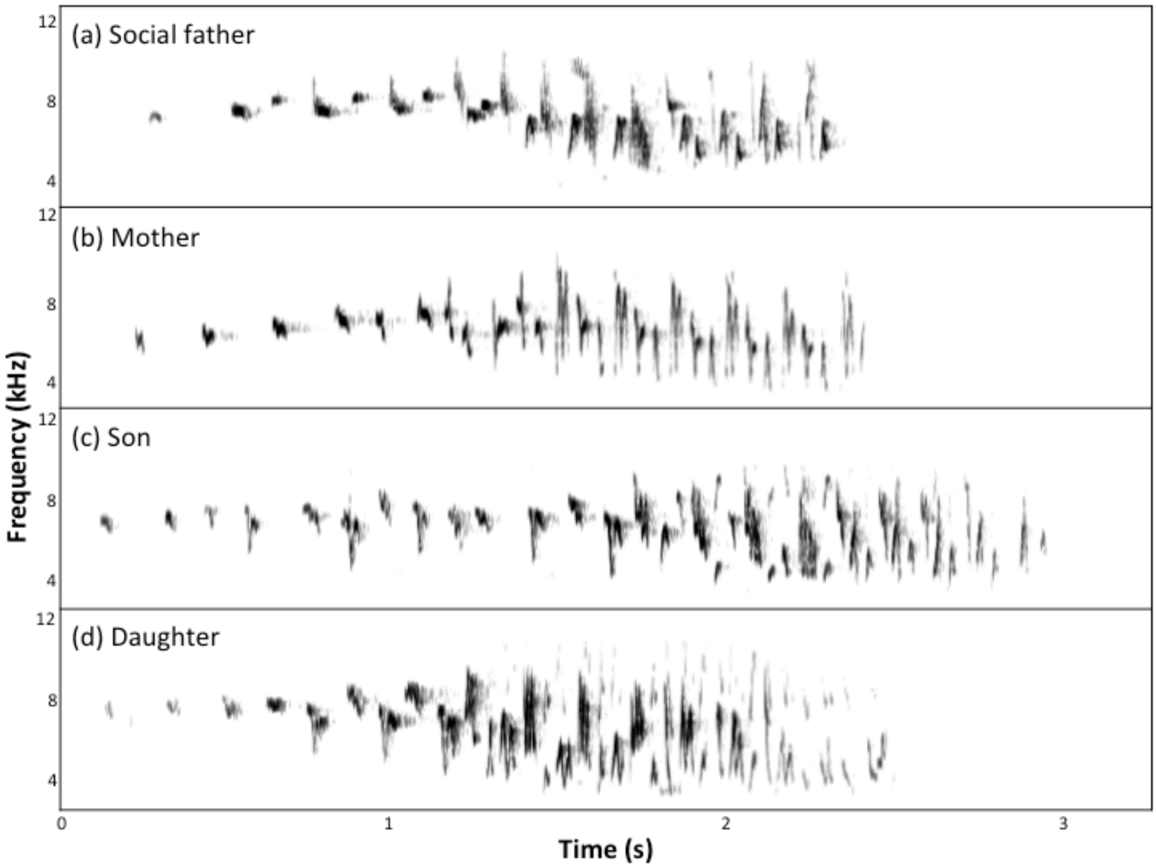

Figure 2.2 Spectrograms of chatter song of a family group of superb fairy-wrens: (a) social father; (b) mother; (c) son, aged nine weeks; (d) daughter, aged 9 weeks.

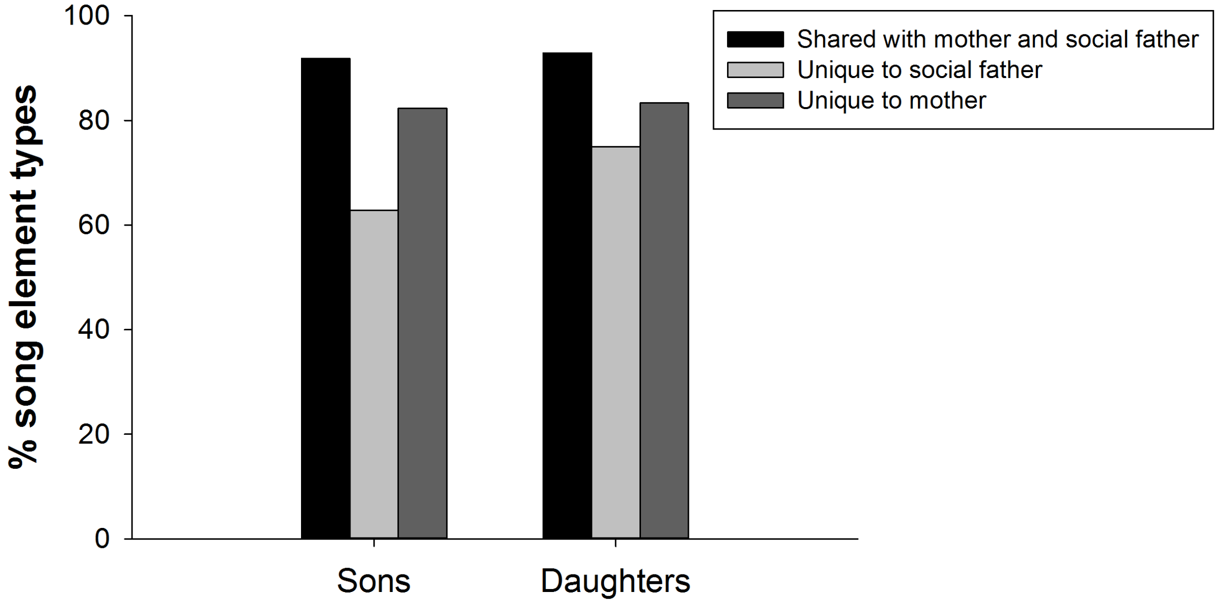

Figure 2.3 The mean percentage of different song element types in the element repertoires of fledged superb fairy-wren sons (N = 8) and daughters (N = 9). Bars show the percentage of elements in the songs of sons and daughters that were ‘shared’ with the social father and mother, ‘unique’ to the paternal song, or ‘unique’ to the maternal song.

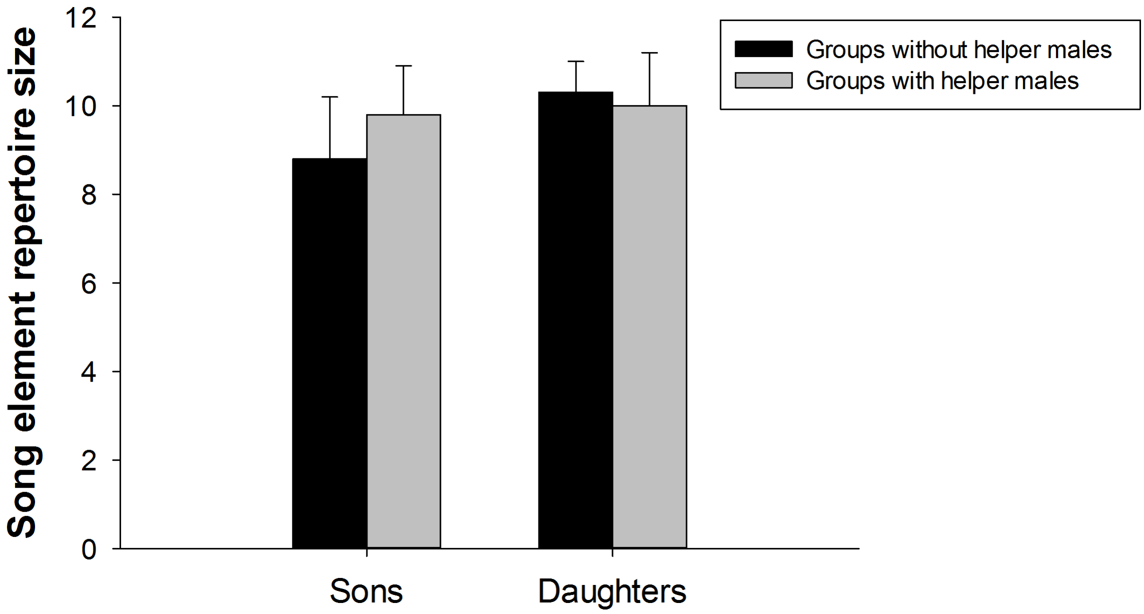

Figure 2.4 The song element repertoire size (number of different element types) (mean ± se) in fledged superb fairy-wren sons (N = 8) and daughters (N = 9). Bars show the element repertoire size in sons and daughters from groups without helper males (social father, mother and brood) and groups with helper males (social father, mother, helper males and brood).

Chapter 3

Cross-fostering shows that superb fairy-wren fledglings acquire song elements through social transmission

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Abstract

Understanding the ontogeny of song learning is important to understand gender differences in adult song. Male and female superb fairy-wrens (*Malurus cyaneus*) produce solo song year round. Here, we use an experimental cross-fostering approach to test if song acquisition among fledgling male and female fairy-wrens occurs through social transmission from social parents or via innate mechanisms from genetic parents. All fledglings produced the 'unique' song elements of their social parents, and no fledglings produced the 'unique' song elements of their genetic parents. There was no correlation between parental song rate and specific song element types in fledglings. The findings support the general consensus that songbirds learn song from singing birds that act as vocal tutors; what is novel about this study is that it uses cross-fostering in wild birds in which both daughters and sons acquire song elements form their social mothers and fathers.

Introduction

In songbirds, song development involves innate and learned pathways that lead to the production of species-specific song (Brainard and Doupe, 2002; Beecher and Brenowitz, 2005; Catchpole and Slater, 2008). Cross-fostering experiments have played a pivotal role in disentangling these two ontogenetic pathways. As evidence of learned song, interspecific cross-fostering studies have shown that male songbirds can learn and produce heterospecific song elements in some species (Eales, 1987; Clayton, 1988; Johannessen et al., 2006; Eriksen et al., 2009). However, most species have an innate predisposition to learn and recognise conspecific song (Marler, 1970; Konishi, 1985; Marler and Peters, 1988). The general consensus is that male songbirds learn their songs and possess an innate template for the development of species-specific song. Early developmental conditions and social factors affect male song learning within species, which has been shown with intraspecific cross-fostering (Buchanan et al. 2004; Gil et al., 2006). Clearly, cross-fostering is a useful experimental approach for understanding social influences and genetic control during song development. However, experimental cross-fostering approaches have not yet been used to test song learning in systems where males and females sing. We use this approach in our study to examine song learning in a wild population of superb fairy-wrens (*Malurus cyaneus*).

During the 1950s to 1970s, studies of song learning done on male birds often used ‘tape tutor’ experiments that deliberately excluded social factors until it was discovered that young birds learn better from live tutors than from tape-recorded song (Thorpe, 1958; Marler, 1970; Todt et al., 1979; Baptista and Petrinovich, 1984, 1986; Marler and Peters, 1988; Beecher, 1996; Beecher and Burt, 2004). There is now strong support for social factors facilitating song learning and tutor preference (Immelmann 1969; Payne 1981; Clayton 1987; DeWolfe et al. 1989; Beecher and Burt, 2004). The parental bond shaped by food provisioning encourages young males to preferentially learn songs of their social fathers that have fed them (Immelmann, 1969; Böhner 1983; Eales 1985; Roper and Zann, 2006). These findings suggest that the social bond between tutor and pupil is a key social influence that affects song learning. Alternatively, the preference for imitating paternal song could be a consequence of passive selection of a song tutor from the nearest adult singer, as paternal song may be the loudest and most frequently heard song (but see Böhner, 1983; Roper and Zann, 2006). However, some species only need limited exposure to learn and imitate tutor song: nightingales (*Luscinia megahynchos*) imitated song phrases heard only 15 times (Hultsch and Todt, 1992), and song sparrows (*Melospiza melodia*) can learn song heard only 30 times (Peters et al., 1992). Nonetheless, the amount of song produced by a potential tutor can influence the pupil's choice of tutor and copy accuracy (Kroodsma and Pickert, 1984; Petrinovich, 1985; discussed in Nelson, 1997). The effect of tutor song rate on song learning in young birds has not been investigated in species where both sexes sing, and hence where both sexes are potential vocal tutors.

In light of recent evidence that song is phylogenetically widespread in extant female songbirds (Odom et al., 2014), there is increasing research focus on the ontogeny of female song (Riebel, 2003; 2016). Currently, few studies have shown that song is socially acquired from both sexes; young birds learn song from both male and female vocal tutors in audio-taped tutored Northern cardinals (*Cardinalis cardinalis*; Yamaguchi, 2001) and blue-capped cordon-bleus (*Uraeginthus cyanocephalus*; Geberzahn and Gahr, 2013; Lobato et al. 2015). In the superb fairy-wren, sons and daughters produce a composite song with element types of social mothers and fathers (Evans and Kleindorfer, 2016). Red-backed fairy-wren (*M. melanocephalus*) offspring produce the vocal elements of their mother (Dowling et al., 2016) but it is unknown whether song elements are socially or innately acquired. Splendid fairy-wren (*M. splendens*) sons learn songs from their social fathers (Greig et al., 2012), but song transmission in females has not been investigated.

Superb fairy-wren males and females produce solo 'chatter' song year round; one function of the chatter song is resource defence (Cooney and Cockburn, 1995; Kleindorfer et al. 2013b; Cain and Langmore, 2015). Within pairs, males and females sing a combination of 'shared' element types produced by the male and female, and 'unique' element types produced by only the male or female within the pair (Evans and Kleindorfer, 2016). This species lives in social groups whereby all adults sing and provision to the brood. Social interactions may play a role in the acquisition of different element types since all group members (social mother, social father, male helpers) and neighbours are potential vocal tutors (Evans and Kleindorfer, 2016). Male and female song rates fluctuate throughout the year, and peak during the start of the breeding season, when individuals are establishing territories and females are fertile (Cooney and Cockburn, 1995; Kleindorfer et al., 2016; but see Cain and Langmore, 2015). Song rates vary considerably among individuals but within pairs, male and female song rates are positively correlated during the fertile period (Cain and Langmore, 2015; Kleindorfer et al. 2013b; Kleindorfer et al., 2016). Females sing less frequently than males during incubation and chick feeding because females that sing near the nest risk attracting nest predators (Kleindorfer et al., 2016). Therefore, superb fairy-wrens show individual variation and sex differences in singing behaviour.

We look at the acquisition of song element types by superb fairy-wren fledglings in a natural, sedentary population where song development and production occur prior to dispersal. We use cross-fostering to test if song elements are learned from social parents or are innately acquired from genetic parents. As song is predominantly learned via imitation of song from vocal tutor(s) in oscine birds, including species where males and females sing (Yamaguchi, 2001; Geberzahn and Gahr, 2013; Lobato et al. 2015), we predict that element types are socially acquired. Given that sons and daughters produce song element types of both parents (Evans and Kleindorfer, 2016), and vocal tutor song output can affect song learning (Kroodsma and Pickert, 1984; Petrinovich, 1985; Nelson, 1997), we also test if the parental vocal tutor song rate predicts (1) the occurrence of within-pair ‘unique’ song element types in offspring song and (2) the complexity of offspring song. We predict that fledglings produce more ‘unique’ element types, and have greater song complexity when social parents have a high song rate. To our knowledge, this is the first study to use intraspecific cross-fostering in a natural population to separate innate and learned pathways for song acquisition in a system with male and female solo song.

Materials and Methods

Study species and field sites

The superb fairy-wren is a sedentary, territorial Southern Hemisphere passerine (Rowley and Russell, 1997). Either socially monogamous pairs or cooperative social groups composed of a single breeding female (dominant female), her social mate (dominant male), and one or more subordinate auxiliary (helper) males occupy a territory year-round (Rowley, 1965; Mulder et al., 1994; Rowley and Russell, 1997). All birds provision the brood and defend the permanent territory (Rowley, 1965; Mulder et al., 1994; Dunn et al., 1995; Rowley and Russell, 1997; Cockburn et al., 2008). Females seek out copulations with extra-pair males from up to 5 territories away, and 75 - 95% of broods contain young sired by extra-pair males (Mulder et al., 1994; Dunn and Cockburn, 1998; Cockburn et al., 2003; Colombelli-Négrel et al., 2009). The breeding season is between August and February. Females build a domed nest and incubate a clutch of 2-3 eggs. Eggs hatch after approximately 14 days of incubation, and nestlings fledge after approximately 12 days in the nest (Colombelli-Négrel and Kleindorfer, 2009). Fledglings are dependent for approximately 30 days but remain in the natal group for several months as independent juveniles (Mulder, 1995; Rowley and Russell, 1997). Males are philopatric and help rear subsequent broods whereas females disperse 1-10 km from the natal population (Rowley, 1965; Cooney and Cockburn, 1995; Mulder, 1995; Cockburn et al., 2003; Double et al., 2005). Nest predation is high (up to 74%) and superb fairy-wrens will have several breeding attempts (1-5) per year (Rowley and Russell, 1997; Colombelli-Négrel and Kleindorfer, 2009). Fledglings also suffer from high mortality, resulting in low rates of reproductive success (Rowley, 1965; Cockburn et al., 2008).

Chatter song (Type I song) is the most commonly produced song of the superb fairy-wren, which males and female sing throughout the year for intrasexual resource defence (Cooney and Cockburn, 1995; Kleindorfer et al. 2013; Cain and Langmore, 2015; Cain et al., 2015). The chatter song is a complex song composed of approximately 8 structurally distinct element types produced approximately 50 times per song for a duration of approximately 3 seconds (Langmore and Mulder 1992; Kleindorfer et al., 2013b). The sensitive phase for learning chatter song is unknown; fledglings begin singing from 3 - 4 weeks after fledgling, and one-year old birds sing full adult song (Rowley, 1965; Langmore and Mulder, 1992). Here, we study the ontogeny of chatter song in male and female superb fairy-wren fledglings. We observed the chatter song rate of 36 adult birds and recorded chatter song of 45 adults and 22 fledglings from September to January 2012 -2014 at Cleland Wildlife Park and surrounding Cleland Conservation Park (34°58’S, 138°41’E) in the Mount Lofty Ranges, South Australia.

Nest Monitoring

For this study, we monitored a total of 136 superb fairy-wren nests over three years during each breeding season. Nests were monitored every 2-4 days to check the status of the nest (building, eggs, nestlings, fledged) and nesting outcome (eggs depredated, eggs abandoned, nestlings depredated, nestlings abandoned, nestlings fledged). Of these 136 nests, 84 were depredated (62%), and 34 nests produced fledglings (25%). We cross-fostered 26 clutches; 20 nests were depredated, and six nests produced nine fledglings. In total, we recorded songs of 22 fledglings from 17 nests (6 cross-fostered nests, 11 natural nests). For 12 of these nests, we also scored the number of chatter songs per hour (songs scored per hour divided by 60 to estimate songs per minute) when fledglings were 3-6 weeks old, which is the age when fledglings begin to sing subsong. Observations were done between 0800 and 1200 hours; observers were approximately 10-20 m from the group. We had multiple song rate observations for three groups so the average song rate was analysed. The majority of individual birds were identified by their unique combination of plastic colour bands. Of the 45 adult birds for which we have song recordings, we colour banded and measured 28 birds using target mist-netting. Each captured individual was marked with a unique combination of plastic colour bands and a numbered aluminium band provided by the Australia Bird and Bat Banding Scheme (banding authority number 2601). All nestlings were banded 7-8 days after hatching. The identity of unbanded birds was determined based on behavioural observations (e.g. number of adults provisioning young, interactions with banded birds) and weekly monitoring of group size and composition. No groups contained more than one unbanded bird of the same sex.

Song recordings and analysis

We recorded and analysed the chatter songs of 67 birds for this study. We recorded adult birds opportunistically throughout the breeding season. We recorded the subsong of fledgling birds at a distance of 5-15 m from the bird every 14 days for 8 weeks post-fledging. Recordings were made with a Telinga Twin Science parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Device 722 digital audio recorder (Sound Devices, USA). Sound files were recorded as broadcast wave files (24 bit, 48 kHz). We transcribed all sound files to a Mac Pro (Apple Inc, USA) for editing with Amadeus Pro 2.1.2 (HairerSoft, Switzerland) and analysis with Raven Pro 1.5 (Charif et al., 2010). Spectrograms were created for 3-5 songs per individual using the Hann algorithm (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DTF 256 samples). For each spectrogram, we scored the number of total elements and the number of different element types. We determined the number of ‘unique’ and ‘shared’ element types for individuals by comparing the observed song element repertoire of social mothers and fathers, and social and genetic parents. The observed song element repertoire was defined as the total number of different element types sung by individuals across the 3-5 songs recorded and analysed. We refer to the song element repertoire of individuals as the ‘observed song element repertoire’ rather than full element repertoire size, which could not be accurately estimated with the number of songs available per bird. We defined ‘song complexity’ as the average number of different element types per song. We identified 16 different song element types in our study populations in South Australia (Evans and Kleindorfer, 2016). We categorised the different element types per spectrogram according to the chatter song element library of Evans and Kleindorfer (2016).

Paternity determination

We carried out the genetic determination of paternity for all fledglings (N = 22) with song recordings in this study. We did this because of the high rates of extra-pair paternity in this species and to ensure we did not cross-foster clutches into the nest of the genetic father. The methodology is outlined in the following steps:

(1) DNA Extraction

In total, 121 samples from 64 males, 28 females and 33 nestlings (complete broods sampled for the 17 nests) were collected for DNA extraction for this study. Blood samples (5 µl) were collected from the brachial vein of each bird and stored on FTA® card (Smith and Burgoyne, 2004). DNA was extracted from blood following a modified version of method 4 for nucleated erythrocytes for use in polymerase chain reaction (PCR) (Smith and Burgoyne, 2004). Specifically, discs of 2 mm^2 were cut from the FTA card, washed for 30 min in 200 µl of FTA lysis buffer (100 mM Tris, 0.1% SDS), then washed for 10 min in 200 µl of DNAzol®. Next, discs were washed 3 times for 5 min with 200 µl of H_2O, then washed for 10 min with 200 µl of 95% ethanol, discarding the solutions after each wash. Samples were dried at room temperature then eluted with 50 µl of TLE buffer, incubated at 90°C for 5 min.

(2) Microsatellite amplification and genotyping

121 individuals were genotyped at nine polymorphic microsatellite loci using primers previously developed for *M. cyaneus* (Mcyu3, Mcyu4, Mcyu5, Mcyu6, Mcyu7, Mcyu8) (Double et al. 2005) and *M. splendens* (Msp4, Msp6, Msp10) (Webster et al. 2004). The full genotyping and parentage analysis protocols used here and in superb fairy-wrens in general have been described in detail elsewhere (Double et al., 1997; Double and Cockburn, 2000, 2003; Beck et al., 2003). We used the multiplex-ready technology (MRT) method to fluorescently label primers during PCR. We used four 5’ labelled fluorescent tags: FAM (GeneWorks); NED, PET, or VIC (Applied Biosystems). We performed PCR amplification (in 12 μl volumes) with PCR reagents in following final concentrations: 1 x μM MRT buffer, 0.2 μM of each primer, 10 μM reverse primer, 10 μM tag forward primer, 0.5 units Immolase, and between 10 and 100 ng DNA. PCR conditions were an initial denaturing step at 95°C for 10 min, followed by 5 cycles of 92°C for 60 s, 50°C for 90 s, 72°C for 60 s, then 20 cycles of 92°C for 30 s, 63°C for 90 s, 72°C for 60 s, and then 40 cycles of 92°C for 15 s, 54°C for 60 s, 72°C for 60 s. The program was completed with a final run of 72°C for 10 min, then terminated at 25°C. The PCR products were pooled, and capillary electrophoresis (ABI 3770 automated sequencer; Applied Biosystems) was used to separate and analyse PCR multiplexes at the Australian Genome Research Facility Ltd, Adelaide. Each 96-well plate contained one column of 8 repeated individuals to account for potential genotyping error. Genotypes were scored using GeneMapper® Software 4.0 (Applied Biosystems) with manual editing by C. E. under the supervision of M. G.

(3) Locus characteristics

We tested each locus for sex linkage using CERVUS version 3.0.3 (Field Genetics Ltd); Hardy-Weinberg Equilibrium (HWE) and linkage equilibrium using GenePop version 4.2 (Raymond and Rousset, 1995). The presence of null alleles, scoring errors, and large-allele drop out was assessed using Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004). No loci consistently showed evidence of scoring errors, stuttering or large-allele dropout. Tests of HWE showed that two loci (Mcyu3, Mcyu6) showed significant departure from HWE for three temporal populations (2012, 2013, 2014; Table 3.1). The remaining six loci conformed to HWE for the three temporal populations, except for four loci (Mcyu4, Mcyu7, Mcyu8, Msp10), which significantly deviated in one temporal population (2013). Tests for linkage disequilibrium showed that two loci (Mcyu6 and Msp10) were significantly linked for the three temporal populations. The locus Mcyu3 showed evidence of null alleles. There were no sex-linked loci. Given that when we analysed maternity of offspring with known mothers, the inclusion of all nine loci produced the lowest error rate and that the loci have been used successfully for South Australian populations of superb fairy-wrens in previous studies (Colombelli-Négrel et al., 2009; Dudaniec et al., 2011), we proceeded with data analysis using all nine loci. The number of alleles per locus across all individuals ranged from 9 - 34 (mean 16.67 ± 2.73 SE), expected heterozygosity ranged from 2.13 – 6.54 (mean 3.81 ± 0.50 SE). Missing data were 0 - 10% across loci, which was calculated using GenAlEx 6.5 (Peakall and Smouse, 2006).

(4) Paternity assignment

Paternity determination was assessed using CERVUS version 3.0.3 (Field Genetics Ltd). We set a minimum confidence level of correctly assigning paternity to 80% and used a likelihood-based approach to analyse the genotypic data (Marshall et al., 1998; Colombelli-Négrel et al., 2009). The natural logarithm of the likelihood ratio is termed the LOD score. The simulation program within CERVUS was used to estimate the critical difference in LOD scores. For the simulations of maternity and paternity, we calculated that 97% of loci were typed and assumed 1% rate of typing error. For each offspring, we analysed parentage based on all candidate parents with positive LOD scores. First we assigned maternity of all offspring to confirm the identity of genetic mothers. We then analysed the paternity of offspring with known mothers (see Colombelli-Négrel et al., 2009). In this study, we only included fledglings where the genetic father could be confidently assigned based on the LOD scores.

Paternity determination of young from cross-fostered clutches and natural nests

None of the fledglings (N = 9) from the six cross-fostered clutches were the genetic offspring of the ‘foster’ male. Therefore, the possible sample size for related and unrelated adults that could have affected song in the 9 fledglings was 12 unrelated foster parents (from the 6 cross-fostered clutches) and 15 genetic parents (from the 6 source nests plus 3 males that were the extra-pair fathers for some of the offspring). At natural nests, 5/11 nests had an extra-pair fledgling sired by a male that was not the social father; these nests were excluded from analysis because of possible conflicts between genetic and social effects on song development. Six natural nests had fledglings that were the genetic offspring of the social male; these nests were included in the analysis that compared the element types produced by fledglings of unrelated cross-fostered nests versus natural nests where both social parents were also the genetic parents.

Statistical analyses

We used IBM® SPSS® 22 for statistical analyses (SPSS Inc., Chicago, USA). We examined the data for normality and homogeneity of variance. The variable ‘percentage of within-pair female unique element types produced by fledglings from cross-fostered nests’ was not normally distributed so the non-parametric alternative was used. The variable ‘within-pair unique element types’ for social fathers and genetic fathers was square-root transformed to satisfy the Shapiro-Wilk test ($P > 0.05$) for normality.

Results

Are song element types socially or genetically acquired by fledglings?

Song characteristics in foster versus genetic parents of cross-fostered nests

Foster parents (N = 12) shared 7.78 ± 0.49 element types with genetic parents (N = 15). Foster parents and genetic parents had 4.22 ± 0.62 unshared element types, which were ‘unique’ to either the foster parents or the genetic parents.

‘Unique’ parental element types at natural and cross-fostered nests

The number of within-pair ‘unique’ element types was comparable in attending females at natural (2.00 ± 0.52, N = 6) and cross-fostered (3.17 ± 1.08, N = 6) nests (independent samples t-test: $t_{10} = -0.976$, $P = 0.352$). Similarly, the number of within-pair ‘unique’ element types was comparable in attending males at natural

(2.33 ± 0.24) and cross-fostered (1.17 ± 0.35) nests (independent samples t-test: t_{10} = 1.637, *P* = 0.133).

Fledglings acquire 'unique' song elements from attending parents

In support of the idea that fledglings acquire song through social learning, all fledglings acquired 'unique' song elements from their social mothers and fathers and not from their genetic parents. Of the six cross-fostered clutches, all fledglings (N = 9) produced 1.17 ± 0.35 'unique' elements from the foster parent and zero 'unique' elements from the genetic parents (χ^2 (1) = 11.455, *P* = 0.002).

Fledgling song contained a comparable number of 'unique' elements from the attending female at natural (1.83 ± 0.40. N= 6) and cross-fostered (2.17 ± 0.87, N = 6) nests (Mann-Whitney U-test: U = 17.500, z = -0.084, *P* = 0.937; Figure 3.1). Fledgling song contained a comparable number of 'unique' elements from the attending male at natural (1.42 ± 0.58) and cross-fostered (0.83 ± 0.40) nests (Mann-Whitney U-test: U = 13.500, Z = -0.744, *P* = 0.485; Figure 3.1).

Does song rate predict the percentage of social mother and social father 'unique' song element types, and fledgling song complexity?

Song rates of the social mother and the social father

Song rates (songs per minute) of social mothers (0.23 ± 0.04) and fathers (0.25 ± 0.04) were comparable when fledglings were 3-6 weeks old (paired samples t-test: t_{12} = 0.425, *P* = 0.678). There was no significant correlation between male and female song rates within pairs (pearson correlation: r = 0.224, N = 12, *P* = 0.461).

Associations between parental song rate and fledgling song complexity

Parental song rate did not covary with the presence of ‘unique’ song elements in the fledglings’ songs. There was no significant correlation between the song rates of social mothers and the percentage of social mother ‘unique’ element types produced by broods (pearson correlation: r = 0.052, $P = 0.867$), or between the song rates of social fathers and the percentage of social father ‘unique’ element types produced by broods (pearson correlation: r = 0.480, $P = 0.160$). There was also no significant correlation between the song rate of the social mother, social father, or combined parental song rate, and the song complexity of fledglings (pearson correlation: all $P > 0.05$).

Discussion

Using cross-fostering, we showed that fledgling fairy-wrens produced song element types ‘unique’ to the songs of their social parents rather than their genetic parents. Furthermore, parental song output did not predict the percentage of ‘unique’ element types produced by fledglings, nor did parental song output influence fledgling song complexity at natural and cross-fostered nests. These findings show that (1) song element types are socially acquired rather than innately acquired, and (2) that parental song output may not be a key social factor for learning complex song in the superb fairy-wren. To our knowledge, this is the first study using an intraspecific cross-fostering design to show that male and female song elements are acquired through social transmission in a wild population.

Our results are in accordance with the large body of research on male songbirds demonstrating that the majority of species socially acquire song by imitating songs of vocal tutors (Baptista, 1996; Catchpole and Slater, 2008). These findings also corroborate similar patterns found in the few other studies that examine song learning in systems where both sexes sing. Geberzahn and Gahr (2013) showed that juvenile blue-capped cordon-bleus exposed to live tutors of both sexes learned song from both adult male and female tutors. Northern cardinals exposed to tape recorded male and female song also learned song from both sexes (Yamaguchi, 2001). Although we show that song elements were socially acquired, we cannot confirm that fledglings learned song elements solely from their social parents because other individuals such as auxiliary helpers or neighbours may have also been vocal tutors. For instance, helpers of the natal group sing the same element types as the social parents (Evans and Kleindorfer, 2016). Also, social parents may share element types with neighbours (Colombelli-Négrel, 2016), so fledglings may have learned from neighbouring birds and helpers too.

Social parents are likely to be the primary vocal tutors for song learning in fledglings because fledglings sing whilst still nutritionally dependent on the parents/natal group and remain within close proximity of them during this phase. Therefore, social parents are the nearest available tutors during this period. In other *Malurus* species, adult males learned song characteristics from their social fathers in the splendid fairy-wren (Greig et al., 2012) and adult offspring produced vocal element types of their mother in their crystallised song in the red-backed fairy-wren (Dowling et al., 2016). The biological fitness derived from learning vocal elements from parents, including their element similarity, may inform testable hypotheses for cues used in kin recognition and inbreeding avoidance, as suggested by Evans and Kleindorfer (2016). In a system with high extra-pair paternity, so prevalent in this system, females in particular may benefit from aurally detecting dissimilar elements that would be a cue for unrelated birds.

When closely related birds have a high proportion of shared song elements or high copy accuracy such as in the red-backed fairy-wren (Dowling et al., 2016), element or call matching may function for kin recognition (e.g. Sharp et al., 2005; Sharp and Hatchwell, 2005; 2006), and inbreeding avoidance. This is in line with innate calls acting as vocal cues of kinship in Bell miners (*Manorina melanophrys*; McDonald and Wright, 2011). Therefore, song element sharing could be a cue used for mate choice, so it could be important for females (and males) to learn song (elements) of their natal group or population prior to dispersal. To optimise outbreeding, females should choose a mate who sings a different song or disassortatively pair for element types so that pairs share few element types (Evans and Kleindorfer 2016; but see Chapter 4). However, females may also learn new element types after dispersal from their natal area, during interactions with territory neighbours or during pair formation.

Song output by adult males and females was not identified as a mechanism for the acquisition of element types by fledglings. This has also been shown for zebra finches (*Taeniopygia guttata*; Böhner, 1983; Mann and Slater, 1995), and song sparrows (Peters et al., 1992). Song output may be constrained because of the high predation risk associated with singing (Kleindorfer et al. 2016). Singing is costly for the signal producer because it exposes adults (Møller et al., 2005; 2008) and nest contents (Kleindorfer et al., 2014b; Kleindorfer et al., 2016) to elevated predation risk. In systems where both sexes produce solo song, males and females share predation risk associated with singing (but males may still have higher potential risk because of conspicuous plumage colouration). Despite individual risk being associated with producing solo song, there are potential benefits for offspring: both sexes are potential vocal tutors and hence, offspring are exposed to songs and element types from multiple potential vocal tutors. Given that there is assortative pairing for shared element types in this system (Chapter 4), and the fact that song rate *per se* by a parent or members of the pair did not predict fledgling song complexity (this study), it is possible that costs and benefits of singing vary in relation to the similarity of parental song repertoire when both sexes are (potential) vocal tutors. Alternatively, parental song output may still be a factor for song learning but young birds may only require exposure to a small number of songs for learning, which remains to be tested.

Identifying mechanisms that facilitate song learning remain largely unknown in systems with male and female song production. The *Malurus* genus is a model system to examine patterns of male and female song production and learning because it contains species that sing solo songs, duets and choruses (Cooney and Cockburn, 1995; Rowley and Russell, 1997; Hall and Peters, 2008; Greig and Pruett-Jones, 2008; Greig et al., 2013; Dowling and Webster, 2013; 2016). *M. coronatus* and *M. melanocephalus* males and females sing duets in which non-identical songs overlap in time and frequency (Hall and Peters, 2008; Dowling and Webster, 2013). Males and females are more likely to sing when another group member sings to form a duet than to sing solo song (Dowling and Webster, 2013). There may be lower individual predation risk when singing in a duet rather than alone yet this may adversely affect song learning because there is less overall exposure to song across time. Song learning has been shown in *M. cyaneus* (this study) and *M. splendens* (Greig et al., 2012), call learning has been shown in *M. cyaneus* (Colombelli-Négrel et al., 2012), and call and song imitation has been shown in *M. melanocephalus* (Colombelli-Négrel et al., 2016; Dowling et al., 2016). This study provides another important piece to the puzzle by showing that song learning occurs via social acquisition in this acoustically diverse family – though much remains to be learned about the social and ecological context that may influence the efficacy of copy accuracy, which was not tested.

Conclusion

This study provides the first experimental cross-fostering test of song acquisition in sons and daughters when social mothers and fathers are (potential) vocal tutors. The fact that cross-fostered sons and daughters produced the unique song elements of their social parents and not their genetic parents is evidence that social song acquisition is a mechanism for vocal repertoire composition in wild birds with male and female solo song.

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2601) from the Australian Bird and Bat Banding Scheme. We thank the Australian Wildlife Society, ANZ Holsworth Wildlife Research Endowment Grant, and Birds South Australia for financial support. C.E. was supported by an Australian Postgraduate Award Scholarship.

Tables

Table 3.1 Allele frequencies for nine loci across three sampling periods (2012, 2013, 2014). N = sample size; Na = number of alleles; Ho = expected heterozygosity, He = observed heterozygosity. Loci that depart significantly from Hardy-Weinberg equilibrium are indicated in bold. * = indicates there was evidence for null alleles at this locus (GenePop 4.2; Micro-Checker 2.2.3; GenAIEx 6.5).

Year	Locus	N	Na	Ho	He
2012	**Mcyu3***	**63**	**14.000**	**0.476**	**0.886**
	Mcyu4	69	17.000	0.884	0.887
	Mcyu5	69	11.000	0.841	0.852
	Mcyu6*	**68**	**20.000**	**0.941**	**0.919**
	Mcyu7*	**69**	**18.000**	**0.826**	**0.901**
	Mcyu8	66	12.000	0.742	0.852
	Msp4	70	7.000	0.871	0.810
	Msp6	70	8.000	0.786	0.774
	Msp10	70	8.000	0.857	0.793
2013	**Mcyu3**	**65**	**13.000**	**0.692**	**0.857**
	Mcyu4	**64**	**12.000**	**0.906**	**0.860**
	Mcyu5	66	11.000	0.803	0.861
	Mcyu6	**65**	**15.000**	**0.938**	**0.905**
	Mcyu7	**64**	**21.000**	**0.766**	**0.919**
	Mcyu8	**62**	**11.000**	**0.758**	**0.851**
	Msp4	63	8.000	0.825	0.814
	Msp6	64	8.000	0.703	0.739

	Msp10	**65**	**8.000**	**0.800**	**0.789**
2014	**Mcyu3**	**46**	**10.000**	**0.630**	**0.847**
	Mcyu4	47	11.000	0.979	0.888
	Mcyu5	47	12.000	0.787	0.828
	Mcyu6	**47**	**16.000**	**1.000**	**0.899**
	Mcyu7	47	20.000	0.936	0.927
	Mcyu8	42	12.000	0.714	0.824
	Msp4	43	7.000	0.744	0.804
	Msp6	46	7.000	0.783	0.732
	Msp10	46	7.000	0.848	0.760

Figures

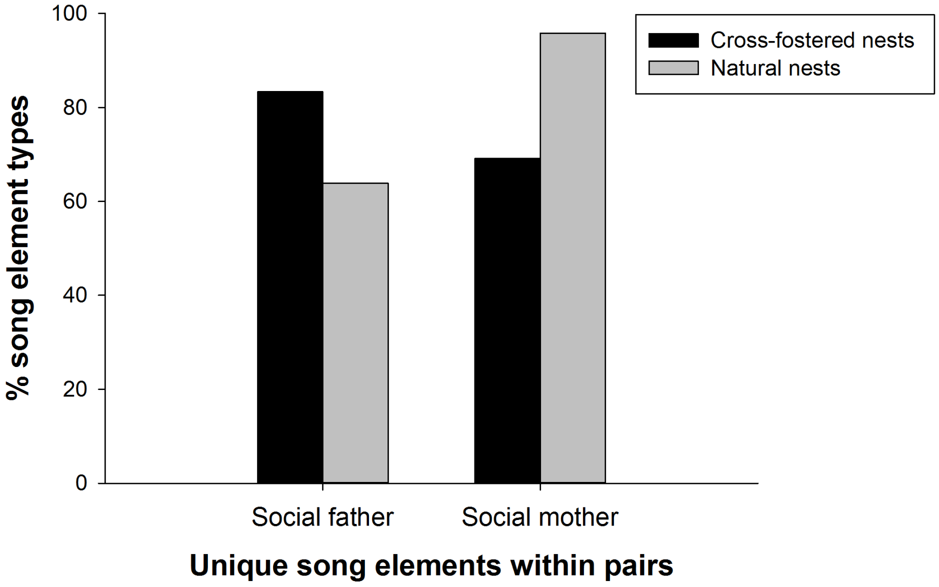

Figure 3.1 The percentage of song elements in fledgling subsong that were also present as 'unique' song elements in song by the social mother and social father. The study was done on superb fairy-wren (*Malurus cyaneus*) fledglings from cross-fostered nests (N = 6) and natural nests (N = 6). Fledgling subsong at cross-fostered nests had zero unique elements from their genetic parents (see Results).

Chapter 4

Solo female song within pairs: assortative pairing for element type

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Abstract

Our knowledge of birdsong as a sexually selected trait mostly stems from systems with male song. In male songbirds, song complexity and repertoire size are honest reflections of individual quality and females have been shown to prefer males with complex song or a large vocal repertoire. We know little about age-effects on song or assortative pairing for song traits in systems with male and female solo song, which we examine in wild superb fairy-wrens (*Malurus cyaneus*). In our study of colour-banded birds across years, there was no difference in song complexity or observed element repertoire size with age. We found significant patterns for song traits within pairs (N = 31): females with a larger observed element repertoire size did not pair with males with a larger observed element repertoire size; rather, these females paired with males that had many shared elements with the female. Females with a smaller observed repertoire size paired with males that produced vocal elements unique to the male. These findings are discussed in relation to possible costs and benefits of pairing for a shared vocal repertoire. We propose that females pair with males with a shared vocal repertoire to minimise predation costs of singing, and maximise benefits associated with fledgling song learning and territory defence.

Introduction

Male songbirds sing to compete with rival males and to attract females (Catchpole and Slater, 2008). Learned features of song can be honest signals of individual phenotypic and genetic quality because song learning and production are constrained by multiple factors (reviewed in Gil and Gahr, 2002). For example, early developmental stress can negatively affect learning and production of complex song (Buchanan et al., 2003; Spencer et al., 2003), because it affects the neural development of the song system, which underpins song learning in passerines (Nowicki et al., 2002; Buchanan et al., 2004). Consequently, females use male song for mate choice because it is a reliable signal of male quality (Gil and Gahr, 2002). Females often prefer males with high song rate (Collins et al., 1994), long song (Kempenaers et al., 1997; Neubauer, 1999), large repertoire size (Eens et al., 1991; Lampe and Seatre, 1995) and complex song (Catchpole, 1980; Mountjoy and Lemon, 1996).

Males with complex song and large repertoire size tend to pair earlier and have higher reproductive success (Catchpole and Slater, 2008). This has been shown for both Northern and Southern Hemisphere male songbirds (Clayton and Prove, 1989; Hiebert et al., 1989; Hasselquist et al., 1996; Buchanan and Catchpole, 1997) but rarely for female songbirds. There is indirect evidence that females that produce more complex song have higher individual quality: female alpine accentors (*Prunella collaris*) that produced complex song had large clutch sizes (Langmore et al., 1996), female European starlings with complex song had repeatable reproductive performance across years (Pavlova et al., 2010), and female New Zealand bellbirds (*Anthornis melanura*) that produced complex songs and sung more frequently had higher fledgling success (Brunton et al. 2016). Female song mainly functions for competition between females for resources rather than mate attraction (Langmore, 1998). There is limited knowledge on whether females maintain a preference for males with longer, more complex song or a large repertoire size, or if females prefer males with similar songs in species with male and female song production.

Positive assortative mating occurs when there is non-random mating between individuals with similar phenotypes (Burley, 1983). Birds assortatively pair for phenotypic traits indicative of individual quality including body size (Delestrade, 2001; Christensen and Kleindorfer, 2007); body condition (Bortolotti and Iko, 1992); age (Potti, 2000; Komdeur et al., 2005); ornaments including bill and plumage colouration (Andersson et al., 1998; Jawor et al., 2003); and call similarity (Moravec et al., 2006). Positive assortative mating may arise by different processes: (1) preferential mating of individuals of similar phenotype if phenotypes vary along a continuum (Burley, 1983); (2) mutual mate choice by high quality individuals, whereby only those of the highest quality can chose mates of similar high quality and low quality individuals have no option but to mate with individuals of equal low quality (Johnstone et al., 1996); (3) intrasexual competition for territories by both sexes in absence of mate choice, if competitive ability is signalled by phenotype and individuals with similar competitive ability share a territory (Creighton, 2001). There can also be negative assortative mating where individuals of dissimilar phenotypes mate more often than expected by chance (Burley, 1983; Houtman and Falls, 1994). Positive assortative mating for ornamental traits can be an effect of pairing for age if ornaments are age-related indicators of quality (Komdeur et al., 2005).

Song is an age-dependent trait when song characteristics change over time. The positive association between song and age can arise when males add elements or songs to their repertoire, and produce more complex song with age (Nottebohm and Nottebohm, 1978; Eens et al., 1992; Lampe and Espmark, 1994; Mountjoy and Lemon, 1995; Gil et al., 2001). This positive relationship between song and age can also arise when males that produce longer, more complex songs or have larger repertoires have higher individual quality and viability, so males that produce short, simple songs or have small repertoires have reduced viability, and are underrepresented in older age classes (Hiebert et al., 1989; Zeh and Zeh 1988). Alternatively, song quality can remain constant across years (Searcy et al., 1985), or decline with age (Marler and Peters, 1981; 1982; DeWolfe et al., 1989). This relationship between age and song can be influenced by developmental patterns of song learning. Passerine species can be close-ended (age-limited) learning species that have a short sensitive phase for song memorization in the first few months of life (Marler, 1970; Eales, 1985) to open-ended learning species that learn songs throughout their lifetimes (McGregor and Krebs, 1989; Espmark and Lampe, 1993; reviewed in Brenowitz et al., 1997; Beecher and Brenowtiz, 2005; Catchpole and Slater, 2008). Our understanding of age effects on song learning and song characteristics are mainly driven by the study of northern temperate species that are often migratory, short-lived, and predominantly males sing (Catchpole and Slater, 2008). In contrast, southern (tropical and southern temperate) species are sedentary and long-lived (Martin, 1996; Russell, 2002; Russell et al., 2002). Males and females sing year-round, and females can produce songs that are equally long, complex, and have a similar repertoire size as males (Brunton and Li, 2006; Pilowsky and Rubenstein, 2013; Schwabl et al., 2015).

We do not yet understand age effects on song in systems with male and female song production, and whether the occurrence of female song influences mate choice in these systems, particularly when recent research suggests that female song is also a reliable signal of individual quality (Langmore et al., 1996; Pavlova et al., 2010; Brunton et al., 2016), and used for mate attraction (Langmore et al., 1996). Keen and colleagues (2016) recently found that song motif diversity increased with age for male and female superb starlings (*Lamprotornis superbus*), but decreased with breeding experience. This suggests that female song, like male song, can also change with age, but it remains unclear if there is assortative pairing for song in species where both sexes sing. We test this in the territorial and long-lived Southern Hemisphere species, the superb fairy-wren (*Malurus cyaneus*).

Male and female superb fairy-wrens produce complex solo chatter song year-round for intrasexual competition for resources (Cooney and Cockburn, 1995; Kleindorfer et al., 2013b; Cain and Langmore, 2015). There is evidence that song is a reliable indicator of individual quality because: (1) females increase song rates and show aggression towards simulated female intruders (Kleindorfer et al., 2013b; Cain and Langmore, 2015); (2) female song rate predicts reproductive success (Cain et al., 2016), (3) female singing behaviour (song rate) is costly due to predation risk (Kleindorfer et al., 2016), (4) female song length (number of elements per song) varies greatly and females that produce longer songs have larger body size (Mahr et al., 2016); and (5) male trill song length increases with age (Langmore and Mulder, 1992; Dalziell and Cockburn, 2008). Furthermore, male song, at least, also functions for mate attraction (Dalziell and Cockburn, 2008; Cockburn et al., 2009). Therefore song may be under sexual and social selection in this species (Dalziell and Cockburn, 2008; Cain et al., 2015; Kleindorfer et al., 2016; Mahr et al., 2016).

In this study we aim to answer three main questions. (1) Do males and females assortatively pair for repertoire size, song complexity, and song length? (2) Do males and females assortatively pair for ‘within-pair shared’ element types and ‘within-pair unique’ element types? (3) Are there age effects on element repertoire size, song complexity, and song length? We investigate these questions by examining songs of social pairs of males and females, and yearling and older birds. We predict that males and females show positive assortative pairing for element repertoire size, song complexity, and song length. We propose that females pair with males that produce different element types to the female (‘within-pair unique’ element types) to increase the element diversity of the pair. Alternatively, males and female may assortatively pair for ‘within-pair shared’ element types, so that females pair with males that produce the same element types. We also predict that older birds have a larger element repertoire size and produce longer, more complex songs than yearling birds.

Materials and Methods

Study species and study site

We monitored a population of superb fairy-wrens at Cleland Wildlife Park and the surrounding Cleland Conservation Park (34°58’S, 138°41’E), located in the Mount Lofty Ranges, 12 km SE of Adelaide, South Australia. It is dominated by open Stringybark woodland with an understory of small trees and shrubs, and areas of open grasslands. This study was conducted during three breeding periods (September – December) in 2012, 2013, and 2014. Individual birds sampled in this study were captured in mist-nets and banded with an aluminium ring provided by the Australian Bird and Bat Banding Scheme, and a unique combination of three colour bands for individual recognition in the field.

The superb fairy-wren is a sexually dichromatic Australian passerine (Rowley and Russell, 1997). Dominant males and females form socially monogamous pairs that occupy a year-round territory (Rowley and Russell, 1997). The cooperative breeding system of this species means that pairs are sometimes aided by up to four subordinate males known as ‘helpers’ that also provision the brood (Mulder et al., 1994; Rowley and Russell, 1997; Cockburn et al., 2008). Social pairs often bond for life, but females may divorce their mate when a better quality territory becomes available or if a son inherits the natal territory after the death of the dominant male (Cockburn et al., 2003). Males are philopatric, remaining in the natal territory as helpers (Mulder, 1995). First-year females disperse 1-10 km from the natal territory to obtain a breeding vacancy (Mulder, 1995; Cockburn et al., 2003). This species is renowned for high levels of extra-pair mating with up to 95% of broods containing at least one extra-pair young (Mulder et al., 1994; Cockburn et al., 2003; Colombelli-Négrel et al., 2009). During the dawn chorus, females seek out males from nearby territories for copulation, preferring older males with early onset of nuptial plumage and longer trill song (Mulder and Magrath, 1994; Dalziell and Cockburn, 2008; Cockburn et al., 2009). In the breeding season, males from neighbouring territories will display their nuptial plumage to females and may carry a yellow petal during courtship display (Mulder and Magrath, 1994; Rowley and Russell, 1997). The breeding season occurs between August and January. Female superb fairy-wrens build the nest and incubate 2- 3 eggs (Colombelli-Négrel and Kleindorfer, 2009). Males help defend the nest and provision the brood (Mulder et al., 1994; Dunn et al., 1995). All individuals defend the territory from intruders (Mulder and Langmore, 1993; Cooney and Cockburn, 1995).

Song complexity and observed repertoire size

We measured chatter song complexity and observed repertoire size in 31 breeding pairs. Chatter song (Type I song) is a complex song composed of different elements, which is produced by males and females year-round (Langmore and Mulder, 1992; Cooney and Cockburn, 1995; Kleindorfer et al., 2013b). Different populations produce different element types (Kleindorfer et al., 2013b). Superb fairy-wrens begin singing chatter song as fledglings, and acquire element types from both sexes via social transmission (Evans and Kleindorfer, 2016; Chapter 3). To identify song complexity, we counted the number of different song element types in the song, which was established by referring to an existing song element library for the study system (Kleindorfer et al., 2013b; Evans and Kleindorfer, 2016). There are 16 different chatter song element types produced by superb fairy-wrens in the study site (Evans and Kleindorfer, 2016). Individuals produce different element types per song, and element types vary between populations (Kleindorfer et al., 2013b; Evans and Kleindorfer, 2016). Adult males sing more complex songs than females in some populations (Kleindorfer et al., 2013b). To test for assortative pairing for complexity, repertoire size, and song length, we examined associations between paired males and females. We test for assortative pairing and not mating, cognizant of high rates of extra-pair copulations in this species. Of the 31 pairs of superb fairy-wrens sampled, six pairs had an unbanded male and eight pairs had an unbanded female, two pairs had both an unbanded male and female. Social pairs and the status of individual birds were identified by behavioural observations throughout the breeding season, including mate guarding of females and onset of male nuptial plumage.

'Within-pair unique' element types and 'within-pair shared' element types

Within pairs, unique element types are elements produced by only the male or the female within the pair, as previously shown by Evans and Kleindorfer (2016). Shared element types are elements produced by both the male and female within the pair. Assortative pairing for 'within-pair unique' element types was assessed by comparing the number of element types produced only by the male and only by the female within the pair. We examined assortative pairing for 'within-pair shared' element types by comparing the percentage of shared element types in the male and female within the pair.

Age and song characteristics

We examined the effect of age on song using individual birds across years, as well as by comparing song across different age cohorts. The sample size for song recordings in colour-banded birds across years was 3 males and 6 females. The sample size for song in different age cohorts was 15 yearling birds compared with 23 birds aged 2 - 3 years. The analysis of song across age cohorts used breeding birds in their first year of brood rearing (referred to as younger birds) and birds one or two years later with at least one year of breeding experience (referred to as older). Age effects on song were examined by comparing element repertoire size, song complexity, and song length between younger birds (young males: N = 8; young females: N = 7) and older birds (older males: N = 13; older females: N = 10). All 38 individual birds sampled for age effects on song were colour-banded. The younger birds had all been colour-banded during the previous year as chicks, and therefore their age was certain.

Song recordings and analysis

We recorded 326 chatter songs from 74 individual birds. We recorded birds opportunistically throughout the breeding season using a Telinga Twin Science parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Device 722 digital audio recorder (Sound Devices, USA). Sound files were recorded as broadcast wave files (24 bit, 48 kHz). We transcribed all sound files to a Mac Pro (Apple Inc, USA) for editing with Amadeus Pro 2.1.2 (HairerSoft, Switzerland) and analysis with Raven Pro 1.5 (Charif et al., 2010). Spectrograms were created for 3-5 songs per individual using the Hann algorithm (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DTF 256 samples). For each spectrogram, we scored the number of total elements and the number of different element types. We defined the ‘observed element repertoire size’ of individuals as the total number of different element types identified in the songs analysed per individual. We were unable to measure the full element repertoire size of superb fairy-wrens in this study due to the limited number of songs analysed per bird. Therefore it is possible that we did not record the complete repertoire size of individuals, and so we refer to the element repertoire size of individuals in this study as the ‘observed element repertoire size’. We defined ‘song length’ as the average total number of element types per song, and ‘song complexity’ as the average number of different element types per song. We categorised the different element types per spectrogram according to the element library of Evans and Kleindorfer (2016).

Statistical analysis

We used IBM® SPSS® 22 for statistical analyses (SPSS Inc., Chicago, USA) for statistical analyses of song characteristics. We examined data for normality and homogeneity of variances. We used paired sample t-tests to compare within-pair male and female observed element repertoire, song complexity, and song length. A single outlier for the variable ‘song length of paired females’ was included in analyses because it was a non-extreme outlier. We used Wilcoxon signed-rank tests to compare ‘within-pair unique’ and ‘within-pair shared’ element types of paired males and females as the data could not be transformed into a normal distribution. Correlations between variables were analysed using Pearson’s correlation for normally distributed data, and Spearman’s rank-order correlation for data that did not meet assumptions of normality or linearity. We used Kruskall-Wallis H tests to compare differences in ‘within-pair unique male’ element types and ‘within-pair shared’ element types between three categorical levels of female song complexity (low: 5.00 – 6.00, medium: 6:01 – 6.99, high: 7.00 – 8.00) and female observed element repertoire size (low: 5.00 – 8.00, medium: 9.00 – 10.00, high: 11.00 – 12.00) because the dependent variables were not normally distributed for all categories. We used Wilcoxon signed-rank tests to compare song characteristics of birds across age because there was a single outlier for the variables ‘song length’ and ‘song complexity’ and three outliers for ‘observed element repertoire size’. These outliers did not affect the significance of the results and are most likely due to the small sample size. To compare song characteristics of younger and older birds, we used independent samples t-tests for normally distributed data that did not contain outliers (observed element repertoire size of younger and older males, song length of younger and older females), and Mann-Whitney U tests for data that were not normally distributed and contained non-extreme outliers (song length of younger and older males, song complexity of younger and older males, observed element repertoire of younger and older females, song complexity of younger and older females).

Results

Assortative pairing for song complexity and observed repertoire size

There were no sex differences for any song characteristics (all $P > 0.1$; Table 4.1), and no significant correlation between either the observed element repertoire size or song length of paired males and females (element repertoire size: $r_s = -0.198$, N = 31, $P = 0.285$; song length: $r_s = 0.129$, $P = 0.490$). However there was a significant negative correlation between song complexity of paired males and females ($r = -0.405$, $P = 0.024$). Females with complex song were paired with males with simple song (Figure 4.1).

Element type, song complexity and observed repertoire size

Each pair had a different combination of unique and shared element types within the pair. Within pairs, males produced 1.84 ± 0.30 ‘within-pair unique’ element types and females produced 1.90 ± 0.32 ‘within-pair unique’ element types; the number of element types did not differ significantly across sexes ($Z = -0.072$, N = 31, $P = 0.943$). Males and females shared 6.39 ± 0.19 element types.

Within each pair, we compared female song complexity and female observed repertoire size with the number of ‘within-pair unique male’ element types. Females with high song complexity and large observed element repertoire size were paired with males with fewer unique element types (female song complexity: $r_s = -0.333$, *P* = 0.016; female observed repertoire size: $r_s = -0.289$, *P* = 0.043). Figure 4.2 shows these results in graphical form for categorical levels of female song complexity (low, medium, high) ($H_2 = 7.215$, *P* = 0.027). The categorical analysis of female observed element repertoire size (low, medium, high) and 'within-pair unique male' elements was also statistically significant ($H_2 = 9.218$, *P* = 0.010). Females with low song complexity were paired with males with many unique elements and females with high song complexity were paired with males with few unique elements (Figure 4.2).

We examined the association between female song complexity and female observed repertoire size with 'within-pair shared male' element types (analysed as shared element types of pairs). The association was not statistically significant for female song complexity ($r_s = 0.100$, *P* = 0.482), but females with larger observed element repertoire size shared many element types with their pair male ($r_s = 0.317$, *P* = 0.031; Figure 4.3).

Age and song

Song characteristics did not change significantly with age. The songs of yearling birds were the same when they were re-recorded in the subsequent year (observed element repertoire size: Z = -0.660, *P* = 0.509; song complexity: z = -0.297, *P* = 0.767; song length: Z = -0.415, *P* = 0.678). There were no significant differences between song characteristics of yearling and older birds (all *P* > 0.05; Table 4.2).

Discussion

Solo female song and complex female song are just beginning to be the subject of intense scrutiny across taxa. Traditionally, complex male song is considered a trait under sexual selection given the honest signalling function of male song complexity and hence direct and/or indirect benefits to females exercising mate choice for male quality. Here, we examined assortative pairing for song complexity in a system with male and female solo song. In 31 pairs, there was a positive association between female observed element repertoire size and the proportion of shared elements with the pair male (pairs had higher element sharing) (Figure 4.3), and negative assortative pairing for song complexity (females with simple song had males with more unique elements) (Figure 4.1, 4.2). These findings are discussed below in relation to possible costs and benefits to females pairing with males with shared or unique element types. Finally, song did not change with age, which we explored with a longitudinal data set using the same colour-banded birds recorded across different years and in a comparison of song characteristics in older versus younger birds.

Costs of female song and benefits of shared vocal repertoire within pairs

One main finding of this study is the pattern of shared element types in pairs with high female repertoire size. Females with small repertoire size and low complexity paired with males with five-fold more unique element types. If observed repertoire size signals individual quality, given previously discussed effects of stressful rearing environments for number of song elements learned, then our study shows that high quality females were paired with males with shared element repertoire and not song complexity *per se*. We discuss the possible costs and benefits of repertoire sharing, but acknowledge that in this study, we measured the observed repertoire size of pairs and not the full repertoire size, so repertoire sharing may increase or decrease with a larger sample size.

Singing is costly when song rate increases predation risk (Møller et al., 2005; 2008). Despite the fact that female song occurs in 32 extant songbird families (71% of surveyed species; Odom et al., 2014), in general females sing less than males (Catchpole and Slater, 2008). When females do sing, they may sing less conspicuously than males. Previous study in superb fairy-wrens has found a cost to vocalization behaviour by females – due to increased nest predation (Kleindorfer, et al., 2014b; 2016). However, there are also benefits to female vocalisation rate including high vocal copy accuracy in offspring in relation to high vocalization rate by attending females (Kleindorfer et al., 2014a). Perhaps high quality females pair with males that have the same vocal repertoire to ensure that the female's memes are passed to the next generation at a lower cost of producing those memes herself. High quality females may pass the high cost of singing (and vocal tutoring) to the pair male. However, our recent work on superb fairy-wren song learning showed that parental song output did not predict the percentage of 'within-pair unique' element types produced by fledglings (Chapter 3). Instead, fledgling fairy-wrens may have higher copy accuracy for song elements if the pupil is repeatedly exposed to the particular song element as a result of multiple tutors producing the same element types. We found evidence of this: fledglings produced a higher proportion of their social parent's 'within-pair shared' song elements than 'within-pair unique' song elements (Evans and Kleindorfer, 2016). Therefore, by pairing with males with shared elements, high quality females could assure that their vocal elements are passed on to their offspring, perhaps at a lower production cost to themselves.

Learning shared parental elements may benefit the offspring in the form of acoustic kin recognition (Sharp et al., 2005). Here, we examined assortative pairing in males and females that were already paired, and assume that females with complex song have higher individual quality than females with simple song. It remains to be tested whether song complexity is a signal of quality in female superb fairy-wrens, and if high quality females are paired first. It should also be investigated whether females with complex song sing less than females with simple song.

Increased survival due to a shared vocal repertoire could arise from more effective territory defence. High quality females may pair with males having shared element types to better coordinate territorial defence. In several songbird species, males share song types or element repertoires with neighbours; the song sharing between males has been shown to be advantageous in male-male competition (Beecher et al., 2000; Wilson et al., 2000; Burt et al., 2001; Marshall-Ball and Slater, 2004). Song sharing enables resident males to acoustically discriminate between neighbours and strangers, and adjust their behavioural response to intruding birds (Wilson et al., 2000; Burt et al., 2001; Beecher and Campbell, 2005). The proportion of song sharing between rival males can influence the intensity and patterns of territorial behaviour (Wilson et al., 2000; Beecher and Campbell, 2005). Several studies have examined song sharing between male and female songbirds within pairs (Hall, 2006; Hall et al., 2015; Marshall-Ball and Slater, 2008; Colombelli-Négrel, 2016). We found superb fairy-wren pairs shared 80% of element types, similar to 84% of shared song types in banded wren (*Thryophilus pleuosticus*) pairs (Hall et al. 2015), and 78% of shared song elements in splendid fairy-wren (*M. splendens*) pairs (Colombelli-Négrel, 2016). Of these three species, female territory defence occurs in superb and splendid fairy-wrens (Kleindorfer et al., 2013b; Colombelli-Négrel, 2016); females do not contribute to territory defence in banded wrens (Hall et al. 2015). We have previously shown that female superb fairy-wrens were the first to respond to female territory intruders (Kleindorfer et al. 2013b). Colombelli-Négrel (2016) found that female splendid fairy-wrens also responded first to female intruders whereas female variegated fairy-wrens (*M. lamberti*) did not. Of these two species, only female splendid fairy-wrens shared more song elements with their social partner than any other male in the population (Colombelli-Négrel, 2016). Both superb fairy-wrens and splendid fairy-wrens have a within-pair shared vocal repertoire and both had a differentiated, and therefore perhaps coordinated and efficient response to intruders (Kleindorfer et al., 2013b; Cain and Langmore, 2015; Colombelli-Négrel, 2016). In studies of coordinated territorial defence, there has been little explicit definition as to what a coordinated response looks like. This needs to be addressed in future research. We suggest that one form of coordinated response is differentiated activity by each pair member (respond to same-sex intruders), and overall a positive correlated response between male and female defence behaviour (Kleindorfer et al., 2013b; Colombelli-Négrel, 2016). Future research could also compare the proportion of territory intrusions and the cost of territory defence in pairs with different levels of element sharing, and in relation to element sharing with their neighbours.

Mechanisms for pair formation based on unique element types: costs of dispersal

In contrast to high quality females with complex song, low quality females with simple song were paired with males that produced more unique element types. We suggest this pattern could arise from female dispersal. Superb fairy-wren females avoid breeding with kin by dispersing from the natal territory, mating with extra-pair males, and divorcing from social mates when sons inherit the male breeder or senior helper position (Cockburn et al., 2003). Breeding vacancies are limited so females disperse up to 10 km to find a breeding position (Mulder, 1995; Cockburn et al., 2003). Dispersal is costly for females because it is energetically demanding, and predation risk is high (Pasinelli et al., 2004; Bonte et al., 2012). Low quality females may need to travel further to obtain a breeding position if there is competition for breeding positions. One issue that dispersing females face is that different populations produce different element types (Kleindorfer et al., 2013b), so females that disperse further are more likely to encounter males with fewer shared element types. Accordingly, low quality females may be forced to pair with males that sing fewer shared elements, and hence these females may have a higher cost of song if low element sharing predicts poor fledgling song learning and poor territory defence.

Chatter song is not age-dependent in males and females

Chatter song characteristics did not change with age for both sexes. Older birds and yearlings had the same repertoire size and produced songs of similar length and complexity (Table 4.2). This suggests that chatter song is not an age-dependent trait, similar to song and repertoire size being independent of age in great tits (*Parus major*; McGregor et al., 1981), song sparrows (*Melospiza melodia*; Searcy et al., 1985), and Darwin's small tree finches (*Camarhynchus parvulus*; Christensen et al., 2006). Chatter song may not be associated with age because young birds accurately imitate songs of high quality adults that have successfully reproduced – their social parents (Evans and Kleindorfer, 2016; see also Greig et al., 2012). Alternatively, the limited age categories that we used (yearlings and older birds) and the song characteristics measured may have diluted effects of age on chatter song, but research by Dalziell and Cockburn (2008), who used a larger range of age categories (from 1 to 7 years) and measured several different song characteristics only found a positive trend for chatter song duration increasing with age in males. Male superb fairy-wrens also sing trill song to attract extra-pair females (Langmore and Mulder, 1992; Dalziell and Cockburn, 2008). In contrast to chatter song, male trill song changes with age. Older males sing songs with a longer trill component, and have higher extra-pair mating success (Langmore and Mulder, 1992; Dalziell and Cockburn, 2008). Based on these findings, high quality females may show social mate choice for males with a shared element repertoire, and show extra-pair mate choice for males for males with longer trill song and earlier onset of nuptial plumage.

Conclusion

Females with larger observed repertoire size paired with males with many 'within-pair shared' elements. Conversely, females with low observed repertoire size paired with males with many 'within-pair unique' elements, generating a pattern of negative assortative pairing for song complexity. The findings of this study raise many questions about the costs and benefits of singing in systems with female solo song, which are just beginning to be explored. The pattern of high element sharing in high quality females is consistent with other studies in males that have found benefits of song and element sharing for improved territory defence. We explore ideas that females may lower the predation costs associated with high song rate by being paired with males with shared vocal repertoire, and increase the benefits of shared vocal repertoire for improved fledgling song learning and better-coordinated territory defence.

Acknowledgments

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Tables

Table 4.1 Song characteristics (mean ± standard error) of pairs (N = 31) of male and female superb fairy-wrens (*Malurus cyaneus*). Statistical results are shown for data that were normally distributed (t-tests) and not normally distributed (Wilcoxon signed rank tests).

Song Characteristic	Male	Female	df	t	z	*P*
Element repertoire size	8.23 ± 0.28	8.29 ± 0.33	30	0.133		0.895
Song length	37.03 ± 1.41	36.00 ± 1.40	30	-0.568		0.574
Song complexity	6.05 ± 0.14	5.96 ± 0.13	30	-0.407		0.687
# unique element types	1.84 ± 0.30	1.90 ± 0.32	30		-0.072	0.943
% shared element types	79.73 ± 3.06	79.66 ± 3.05	30		0.071	0.943

Table 4.2 Song characteristics (mean ± standard error) in relation to age in superb fairy-wrens for yearling males (N = 8) and females (N = 7), and older males (N= 13) and females (N = 10). Statistical results are shown for data that were normally distributed (t-tests) and not normally distributed (Mann-Whitney U-tests).

Sex	Song Characteristic	Yearling Birds	Older Birds	df	t	z	*P*
Male	Element repertoire size	9.00 ± 0.46	8.23 ± 0.28	19	1.580		0.131
	Song complexity	6.25 ± 0.20	5.74 ± 0.13			-1.890	0.064
	Song length	35.18 ± 2.47	38.21 ± 2.17			1.232	0.238
Female	Element repertoire size	7.43 ± 0.92	8.80 ± 0.47			1.777	0.088
	Song complexity	5.34 ± 0.35	6.01 ± 0.26			1.498	0.161
	Song length	31.19 ± 1.78	36.55 ± 3.64	12.73	-1.324		0.209

Figures

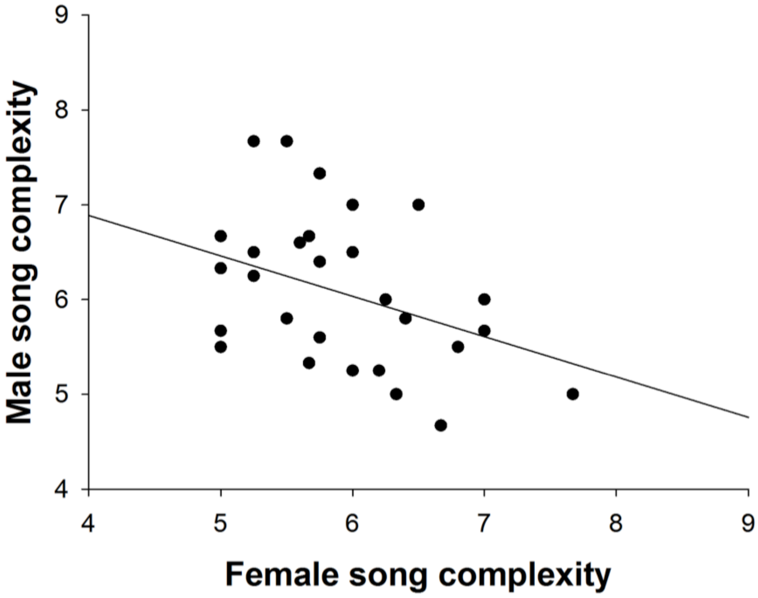

Figure 4.1 The association between pair male and female superb fairy-wren chatter song complexity (N = 31 pairs).

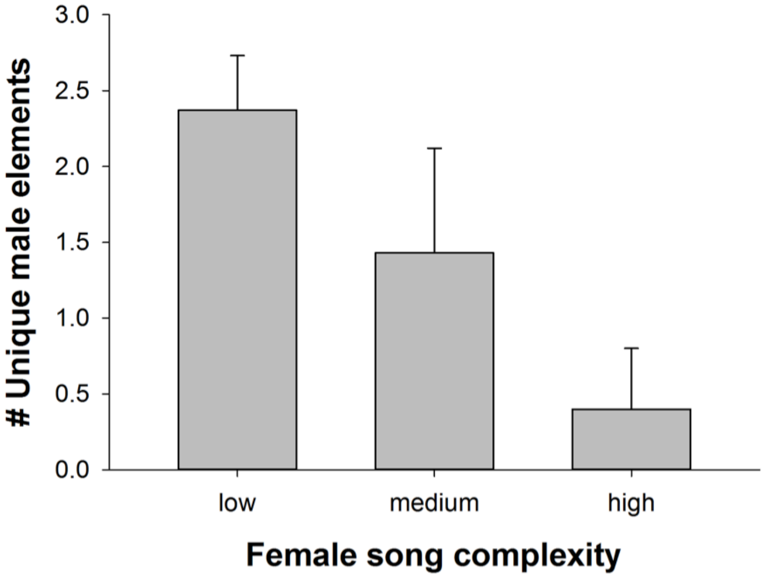

Figure 4.2 The number (mean ± se) of unique male element types for three categorical levels of female song complexity (low: 5.00 – 6.00, medium: 6:01 – 6.99, high: 7.00 – 8.00) for 31 pairs of superb fairy-wrens. Females with low song complexity were paired with males with significantly more unique elements (see Results).

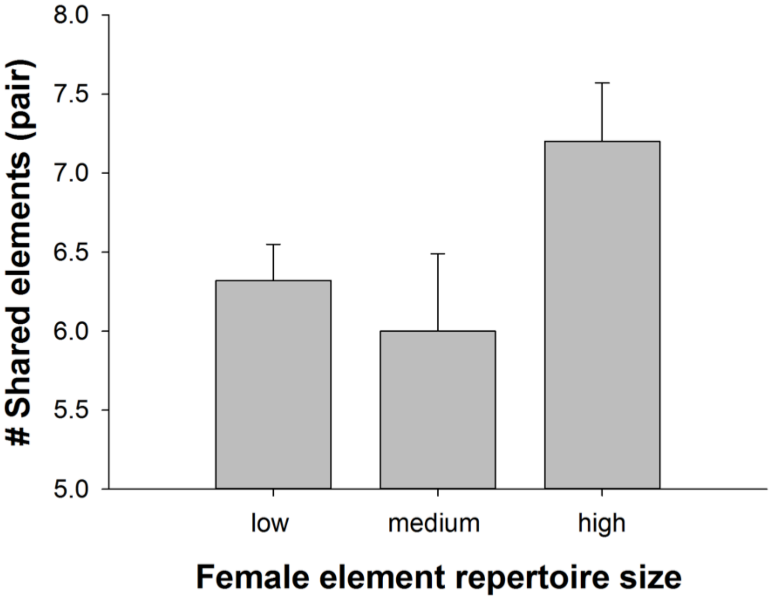

Figure 4.3 The number (mean ± se) of shared element types of paired males and females for three categorical levels of observed element repertoire size (low: 5.0 – 8.0, medium: 9.0 – 10.0, high: 11.0 – 12.0) in females. Data are from 31 pairs of superb fairy-wrens. Females with larger vocal repertoire were paired with males with significantly more shared elements (see Results).

Chapter 5

Female in-nest chatter song increases predation

Sonia Kleindorfer, Christine Evans and Katharina Mahr

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Abstract

Female song is an ancestral trait in songbirds, yet extant females generally sing less than males. Here, we examine sex differences in the predation cost of singing behaviour. The superb fairy-wren (*Malurus cyaneus*) is a Southern Hemisphere songbird; males and females provision the brood and produce solo song year-round. Both sexes had higher song rate during the fertile period and lower song rate during incubation and chick feeding. Females were more likely than males to sing close to or inside the nest. For this reason, female but not male song rate predicted egg and nestling predation. This study identifies a high fitness cost of song when a parent bird attends offspring inside a nest and explains gender differences in singing when there are gender differences in parental care.

Introduction

In songbirds, condition-dependent song is generally considered a sexually selected trait used by males to repel rivals and attract females (Andersson, 1994; Catchpole and Slater, 2008). However, there is growing focus on the occurrence (Najar and Benedict, 2015) and functions of female song (Cain et al., 2015), mostly using the perspective of life history and social selection theory. Female song is widespread and ancestral in songbirds, and females sing across 71% of extant species spanning 32 families (Odom et al., 2014). Many Southern Hemisphere songbirds are sedentary, and females and their pair males sing solo song year-round to defend the territory (Cooney and Cockburn, 1995; Brunton and Li, 2006). Females generally sing less than males. The findings by Odom et al. (2014) raise questions about why some females have lost or gained song, and why many females currently sing less than males. Singing is a variable behaviour and not a fixed trait, and therefore a songbird may increase or decrease its song rate in relation to how it perceives its surroundings, including social and ecological context (Fontaine and Martin, 2006; Zoratto et al., 2014). One approach to understand gender differences in singing behaviour is to test if there are gender differences in the costs of singing, which is the aim of this study.

Our study system is the superb fairy-wren (*Malurus cyaneus*), a sedentary long-lived Southern Hemisphere songbird. Male and female fairy-wrens sing solo ‘chatter’ song across the year (Cooney and Cockburn, 1995), and both sexes defend the territory against intruders (Kleindorfer et al., 2013b). Male and female superb fairy-wrens differ in patterns of parental care: the female is a uniparental incubator and both sexes feed the chicks (Rowley and Russell, 1997). We test if fairy-wren song rate increases nest predation in relation to nesting phase and primary parental care provider. (1) During the fertile period, song rate should be high in both sexes given no nest attendance; (2) during incubation, female song rate should predict egg predation because females are uniparental incubators; (3) during chick attendance, pair song rate should predict chick predation because both sexes feed the young. (4) Both sexes should vocalise away from the nest to reduce predation risk from nest conspicuousness. (5) At artificial nests at which we broadcast female song, we predict higher egg predation when there is a higher rate of song. Prediction (1) could predict either a low song rate at later stages or (4) that both sexes will sing away from the nest. If prediction (4) is correct, then (2) and (3) may not follow; that is, if neither sex sings near the nest, then the song rate of neither sex would matter. If both sexes have the same song rate at the nest, then there should not be an effect of sex-specific song rate on predation.

Materials and Methods

We monitored chatter song rate and nesting outcome at 72 wild superb fairy-wren nests from September to December during 2013 and 2014 at Cleland Wildlife Sanctuary (34°58'S, 138°41'E) and Newland Head Conservation Park (35°37'S, 138°29'E). One nest was analysed per nesting phase: fertile period (N = 20), incubation (N = 26), and chick feeding (N = 26). In 2014, we measured egg predation at 45 artificial domed nests in relation to experimental broadcast of song rate at Scott Creek Conservation Park (35°05'S, 138°41'E).

Male and female fairy-wrens produce a solo chatter song that consists of approximately eight different vocal elements produced approximately 50 times per song for approximately 3 s (Kleindorfer et al., 2013b). Fairy-wrens learn this song as fledglings and produce the song as adults (Evans and Kleindorfer, 2016; Chapter 3). We have previously studied incubation calls in this system. Incubation calls are quieter than chatter songs (approx. 60 dB versus approx. 87 dB at 1 m) and are produced by incubating females while inside the nest; the incubation call consists of two vocal elements repeated approximately 5 times for approximately 1 s

(Colombelli-Négrel et al., 2012; Colombelli-Négrel et al., 2014). In general, songbird songs are learned, have many elements, and are produced by adults; calls have few elements and are produced by all age groups (Price, 1979).

Song rate and predation at natural nests

Territories were monitored every 3 days to record date of first egg, hatching success, predation, and vocalisation behaviour. We scored the number of chatter songs per nesting phase. The fertile period was considered to begin approximately 5 days before egg laying and terminate with egg laying; we scored number of chatter songs per 20 min (multiplied by three to estimate songs per hour) and retrospectively assigned nests after determining date of first egg. All nest observations were done between 07.00 to 10.00. Incubation and nestling phase are each approximately 15 days. We scored number of songs per hour during 1 h of nest observation during either incubation (egg age: 10 - 12 days) or chick feeding (chick age: 2 - 4 days). At 12 nests in 2014, we recorded minimum distance (m) of singer to nest and the proportion of nests at which the female produced chatter song inside the nest. For nest observations, the observer was hidden in vegetation (approx. 15 m from the nest). Given the estimate error for birds singing from vegetation near the nest, we used ‘minimum distance of the singer to the nest’ for statistical analysis. We noted egg and chick predation when nest contents were missing during 3-day nest checks; chicks that survived to 10 days were considered to have fledged.

Song rate and predation at artificial nests

From 20 September to 5 October 2014, we experimentally tested the effect of female song rate on egg predation. Artificial domed nests each baited with one quail egg were placed every 30 m along three transects; each transect was separated by 500 m. For 3 h (07.00 to 10.00) at every nest including control nests, we placed a MoshiTM BassBurger rechargeable portable speaker (sensitivity: greater than 80 dB; frequency response: 280 Hz - 16 kHz) connected to an Apple iPod (Apple Inc., USA) below the nest. At every second nest, we broadcast female chatter song at low song rate (six calls per hour), and at every third nest we broadcast female chatter song at high song rate (20 calls per hour). We saved the playback stimuli as uncompressed 16 bit 44.1 kHz broadcast wav files using Amadeus Pro v. 1.5; playbacks were 85 - 88 dB SLP at 1 m, which is within the natural level. We broadcast chatter song every day for 3 days and analysed predation outcome after 3 days (presented here), as well as 14 days (data available from Dryad). Predation was scored if the egg was missing.

Data were analysed with SPSS 20 for Windows (SPSS Inc., Chicago, USA). The variable ‘number of songs per hour’ was log transformed to satisfy requirements of normality for parametric tests. We confirmed homogeneity of variance prior to using ANOVA.

Results

Natural nests

The number of pair male and female chatter songs per hour was significantly correlated during the fertile period ($r = 0.83$, $N = 20$, $P < 0.001$), but not during incubation ($r = -0.04$, $N = 26$, $P = 0.858$) or chick feeding ($r = 0.33$, $N = 26$, $P = 0.099$) (Figure 5.1). Song rate differed significantly across the three nesting phases (ANOVA: males: $F_{2,71} = 14.22$, $P < 0.001$, partial $\eta^2 = 0.29$; females: $F_{2,71} = 6.07$, $P = 0.004$, partial $\eta^2 = 0.15$). In males, song rate was highest during the fertile period

(23.5 ± 4.7) compared with incubation (11.3 ± 3.1) and chick feeding (4.3 ± 0.9). Female song rate was also highest during the fertile period (17.5 ± 3.8) compared with incubation (8.7 ± 1.3) and chick feeding (6.0 ± 1.4). Using paired t-test with log-transformed data, males sang more than females during the fertile phase ($t = 2.29$, $P = 0.034$), but song rate in males and females was comparable during incubation and feeding (both $P > 0.2$). Males had higher song rate during incubation than feeding (independent t-test: $t = 2.7$, $P = 0.010$); female song rate was comparable between incubation and feeding (independent t-test: $t = 1.68$, $P = 0.099$).

During incubation, high female song rate predicted egg predation (multiple regression: female song rate: $r_{part} = 0.64$, $P = 0.001$; male song rate: $r_{part} = -0.152$, $P = 0.467$) (histograms in Figure 5.2). During the chick phase, high female song rate predicted chick predation (female song rate: $r_{part} = 0.411$, $P = 0.041$; male song rate: $r_{part} = -0.241$, $P = 0.246$) (histograms in Figure 5.2). Total songs per hour per nest was not significantly associated with egg or chick predation (both $P > 0.3$). Females sang significantly closer (m) to the nest (0.7 ± 0.3) compared with males (6.3 ± 0.5 m) (paired t-test: $t = -10.633$, $df = 11$, $P < 0.001$). A higher proportion of females (6/12) sang while inside the nest compared with males (0/12) (Likelihood ratio = 10.357, $P = 0.005$, Cramer's V = 0.477). At all six nests with female chatter song inside the nest, the female produced one chatter song.

Artificial nests

Egg predation was significantly different across treatment groups (Likelihood ratio = 9.834, $P = 0.007$). Egg predation was lowest at control nests (0%), intermediate at nests with low song rate (20%), and highest at nests with high song rate (40%).

Discussion

In this study we show sex differences in the predation cost to singing, which provides a new perspective to test differences in singing behaviour when both sexes produce solo song. The number of female, but not male, songs per hour predicted egg and chick predation at natural nests. Compared with males, female chatter song is shorter (Kleindorfer et al., 2013b), and therefore song characteristics are an unlikely explanation for the observed difference in predation. Females sang significantly closer (m) to the nest than males and were more likely to produce song while inside the nest. Female song likely revealed the nest location to predators (Magrath et al., 2010). Artificial nests at which we experimentally broadcast higher song rate had more egg predation. While our experimental chatter song rate was within the normal range observed for 15 m near the nest, it was higher than that observed for females inside the nest, which could have exaggerated effect size.

Male and female song rate was positively correlated during the fertile period (see also Hall and Peters, 2008) but not during incubation and feeding (Figure 5.1). The change in pattern of association (but no significant difference in song rate between the sexes) suggests different mechanisms and/or functions of song in males and females across the nesting phase (Peters et al., 2013; Chiver et al., 2015). It remains to be tested if females with lower song rate produce more offspring (silent female hypothesis) or if females that adaptively adjust song rate produce more offspring (adaptive female song rate hypothesis). We did not compare song rate in the same bird across nesting phase and cannot comment on singing consistency (Cain and Langmore, 2015). Males and females with eggs and chicks had lower song rate than birds during the fertile phase, and females that sang more incurred more nest predation.

Non-human animals have adaptive risk assessment and attend to aural cues of predators and other brood threats (Blumstein et al., 2008; Chan and Blumstein, 2011; Kleindorfer et al., 2013a). One explanation for different patterns of male and female song rate is that each sex is more likely to encounter different threats while attending the nest. Females have been shown to adjust vocalisation behaviour to aural threats. Previously, our group showed increased in-nest incubation call rate by females experimentally exposed to a brood parasite threat (Kleindorfer et al., 2014a); higher incubation call rate resulted in benefits and costs. Fairy-wren embryos exposed to many incubation calls had higher vocal copy accuracy as chicks and received more parental feeds (Kleindorfer et al., 2014a), parents had improved discrimination of intruder (cuckoo) chicks that did not learn as embryos (Colombelli-Négrel et al., 2012), but nests with many incubation calls had more egg predation (Kleindorfer et al., 2014b). Here, we found that female chatter song rate, similar to female incubation call rate, increased nest predation. It is unknown if fairy-wrens have a capacity for predator risk assessment that affects song rate – but intriguingly, Fontaine and Martin (2006) found that male song rate increased after the experimental removal of nest predators (Fontaine and Martin, 2006).

Solo chatter song likely has multiple functions in fairy-wrens, including territory defence (Kleindorfer et al., 2013b; Odom et al., 2014; Cain and Langmore, 2015 Cain et al., 2015). What is novel about this study is that 50% of nesting females sang chatter song from inside the nest. Females produced song if they happened to be inside the nest when the male sang upon arrival within 15 m of the nest, but females did not initiate song from inside the nest (Kleindorfer per. obs). This raises questions about additional functions of female song (e.g. pair-bond, vocal tutoring). Notably, male only care of eggs occurred in some of the oldest bird lineages (e.g. megapodes, ratites; Cockburn, 2006). Given the high costs of female song under conditions of in-nest parental care, the evolution of avian sociality is creating strong selection on female vocalisation behaviour including, we suggest, selection for cognitive capacity to discriminate and assess predation threats during nest attendance.

Acknowledgements

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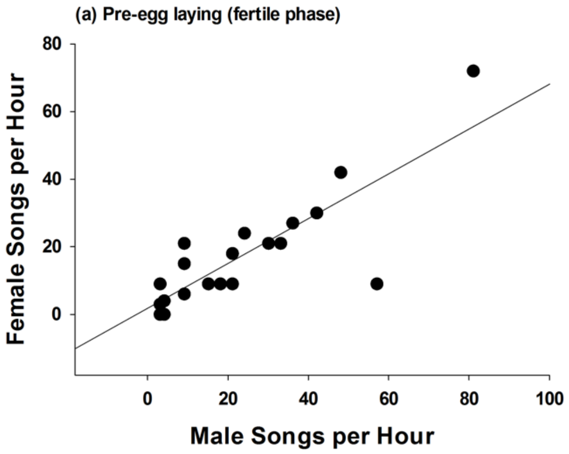
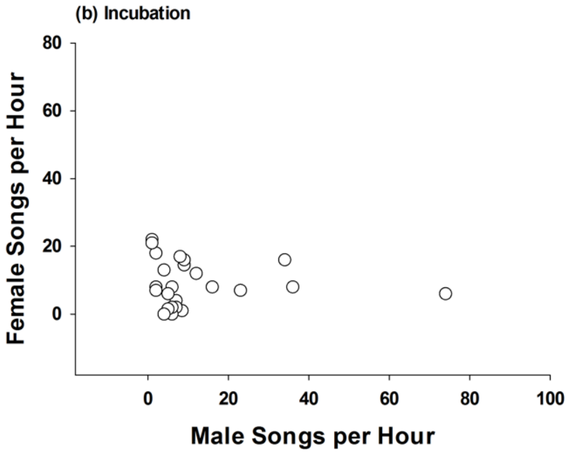
Figures





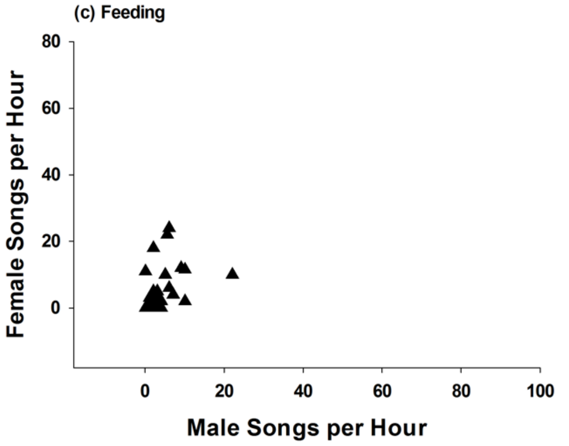
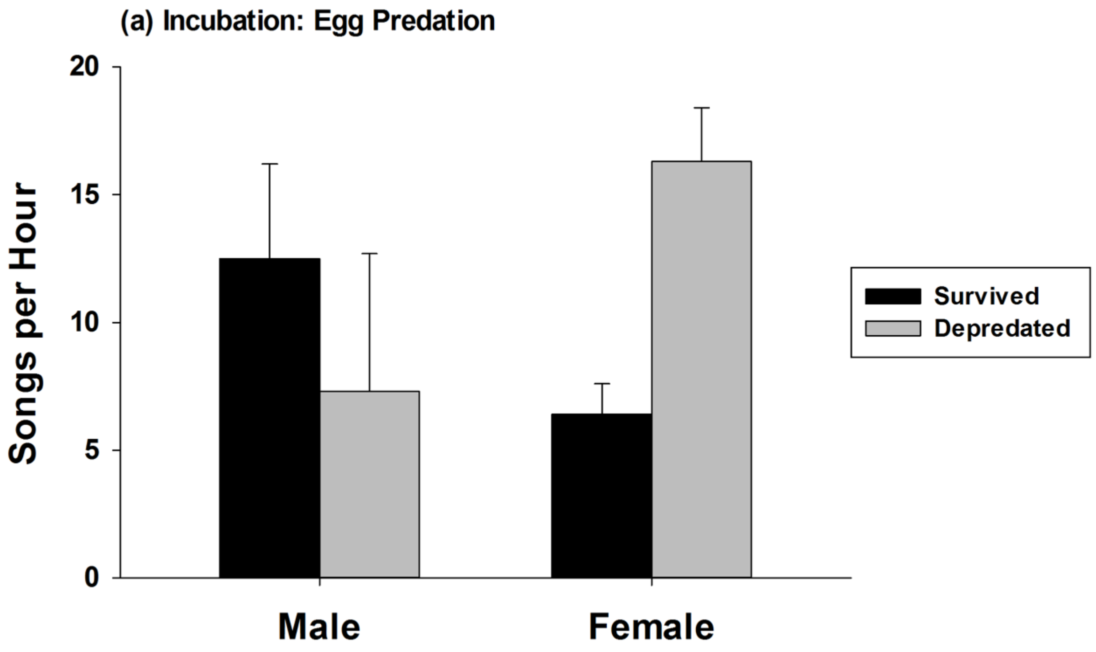


Figure 5.1 The association between pair male and female superb fairy-wren chatter song rate during the fertile (N = 20 nests), incubation (N = 26 nests), and chick feeding (N = 26 nests) phases. Data are independent per nesting phase.



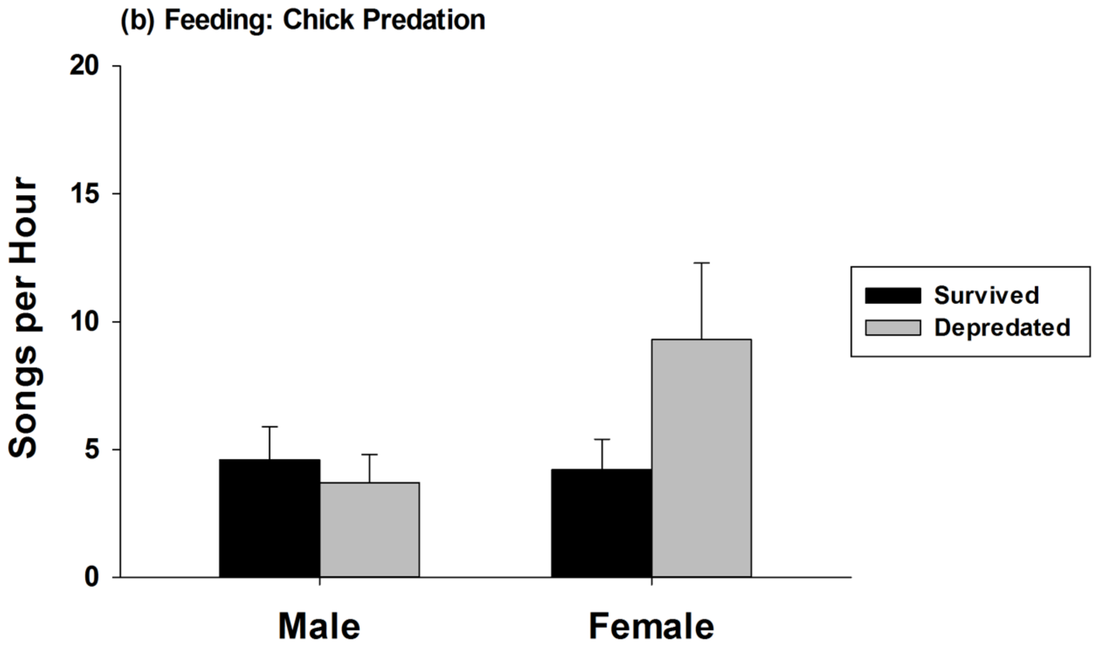


Figure 5.2 The number of chatter songs (mean ± se) by attending male and female superb fairy-wrens in relation to (a) egg predation and (b) chick predation. Female song rate was significantly higher at depredated nests. Male song rate did not predict predation.

Chapter 6

General Discussion and Conclusion

Synthesis of findings & future directions

The emergence of female song as a phylogenetically and geographically widespread trait in oscine passerines requires us to re-evaluate our understanding of the evolution of bird song in males and females. To date, bird song is regarded as one of few examples of animal behaviour where all four categories of Tinbergen's (1963) questions have been addressed in detail (Bateson and Laland, 2013). However it has become apparent that our current understanding of bird song is based almost solely on systems with male song production due to historical and geographical research biases. My thesis adds to the growing body of work on female song by addressing fundamental questions about whether there are differences in song learning in sons and daughters, the role of female song in mate choice, and sex differences in fitness costs of singing behaviour in a system where both sexes produce complex solo song. Here, I will evaluate and discuss the findings of this thesis in the perspective of Tinbergen's levels of analysis for explaining behaviour.

Ontogeny

My thesis shows that superb fairy-wren (*Malurus cyaneus*) sons and daughters produced the song elements of (foster) mothers and social fathers (Chapter 2 and 3), and had comparable song element repertoires as fledglings (Chapter 2). Cross-fostering demonstrated that these element types were socially transmitted from parent to offspring (Chapter 3). Previous research on male and female song learning has also shown that young birds learn and produce similar numbers of elements and songs, and learn songs from both sexes (Yamaguchi, 2001; Geberzahn and Gahr, 2014). These studies and Chapters 2 and 3 suggest that males and females are capable of learning song from either sex, and do not show a preference for same-sex vocal tutors. Instead, both males and females can be vocal tutors. Female superb fairy-wrens are not only vocal tutors for fledglings that learn chatter song, but are the sole vocal tutors for embryos that learn the incubation call in ovo (Colombelli-Négrel et al., 2012). Together, this work illustrates the active role females play in vocally tutoring male and female offspring, and therefore identifies vocal tutoring as a form of maternal investment (Kleindorfer et al., 2014a). Given the costs and benefits of vocal tutoring, and the adaptive flexibility in vocalisation behaviour, the female superb fairy-wren fulfils all three criteria outlined in the operational definition of (non-human) teaching making them one of five non-human species that have been ‘acceptably’ classed as teachers (Kleindorfer et al., 2014a, 2014b; Ridley and Ashton 2015). What my thesis shows is that daughters, and sons, had equal learning (vocal imitation) under the same female teacher (vocal tutor).

In systems with female-biased dispersal, such as in superb fairy-wrens, the subsequent differences in encountered social environments after dispersal may contribute to sex differences and individual changes in adult song, perhaps as the consequence of social song adjustment (Lobato et al., 2015). For example, females could tactically lose vocal elements (to shorten song and reduce the predation cost of singing) if those elements are present in her mate’s song; this would influence the pattern for element sharing within social pairs shown in Chapter 4. Neighbours are also potential vocal tutors since fledglings are exposed to songs of neighbouring birds during song development and song element sharing between social parents and neighbours is likely (Colombelli-Négrel, 2016). Dispersing individuals may also learn from neighbours or change song to match neighbours' songs during territory settlement, as in other species including Bewick's wrens (*Thryomanes bewickii*; Kroodsma, 1974), indigo buntings (*Passerina cyanea*; Payne and Payne, 1993), and Nuttall's white-crowned sparrows (*Zonotrichia leucophrys nuttalli*; Bell et al., 1998). Finally, recent evidence suggests that element types acquired from social mothers during development remain in adult song in the red-backed fairy-wren (*M. melanocephalus*; Dowling et al. 2016). Thus, while there might be some adjustment of vocal repertoire across lifetime, which has not been studied in detail in females, my thesis showed that daughters learn vocal elements and, in line with other studies, most learned elements are maintained throughout adulthood (Chapter 4) as assessed by observed vocal repertoire in adult female song.

Mechanism (causation)

This thesis did not address the mechanism for song learning or production. However, I consider the main thesis findings in the light of causative explanations for song in my study system. Because I measured the same patterns of song element imitation in young males and females (Chapter 2), and the same pattern of song complexity in adult birds (Chapter 4), it is reasonable to infer that the underlying causal mechanism for song learning and production is similar in superb fairy-wren males and females. The neural song system including the brain structures of the higher vocal centre (HVC) that control learning and production of song (Brenowitz and Beecher, 2005), has not been examined in superb fairy-wrens, and therefore not across sexes. This is a next step to understand the mechanisms of song in this model system for solo male and female song. The neural motor pathway that includes the HVC nuclei and robust nucleus of the acropallium (RA) are associated with the repertoire of elements (Nottebohm and Arnold, 1976; Bolhuis and Gahr, 2006). There is some support that species with male and female song with comparable complexity and similar rate have similar HVC and RA volumes (Brenowtiz et al., 1985; Brenowitz and Arnold, 1986). Yet there is accumulating evidence that males and females can produce duets or solo songs of similar complexity despite significant male-biased sexual dimorphism in song system anatomy (Brenowitz and Arnold, 1986; Gahr et al., 1998; 2008; Jawor and MacDougall-Shackleton, 2008; Schwabl et al., 2015). Females can also produce complex song despite having significantly lower androgen levels than males (Schwabl et al., 2015). There is a call to re-examine the relationship between the song system structure, hormonal influences on song, and song complexity and behaviour in systems with male and female song to more closely examine the mechanisms controlling song learning and production in songbirds.

Phylogeny (evolution)

Recent phylogenetic studies showed that song in females is an ancestral trait for modern songbirds (Garamszegi et al., 2007; Odom et al., 2014). The geographical origin of the oldest phylogenetic lineages of songbirds is Australasia (Barker et al., 2004), and female song remains widespread in Australasia (Robinson, 1949; Odom et al., 2014). Female song is less common in northern temperate regions (Slater and Mann, 2004). Accordingly, there must be different selective forces promoting the maintenance of song in female Australasian songbirds, and the loss of this trait in northern temperate songbirds. Identifying these evolutionary pressures is exciting and key to understanding the evolution of female song.

The contrasting life history traits of birds across hemispheres may reasonably contribute to differences in the prevalence of female song. Many Australian species such as superb fairy-wrens are sedentary, highly territorial, and males and females form long-term partnerships (Robinson, 1949; Russell and Rowley, 1997; Russell, 2000; Russell et al., 2002). Year-round territoriality is strongly associated with female song, as many females sing for territory defence and other resources (Morton, 1996; Langmore, 1998; Hall, 2004; Odom et al., 2014; Tobias et al., 2016). Female song is also associated with a tropical distribution, convergent sex roles, reduced sexual dichromatism, and long-term social bonds (Slater and Mann, 2004; Langmore 1998; Hall, 2004, Price, 2009; Odom et al., 2014; Najar and Benedict, 2015; Tobias et al., 2016; Webb et al., 2016). For Australian songbirds, local climatic conditions allow for year-round availability of ecological resources, and hence the sedentary lifestyle of many species. In turn, this supports the formation of long-term social partnerships between males and females. Together, year-round territories and social mates are critical resources worthy of defence by both sexes. Subsequently, females play an active role in resource defence, and sing solo song or duet with their social partner to defend resources in the few Australian species, including fairy-wrens, where female song has been examined (Farabaugh et al., 1992; Hall, 2000; Rogers et al., 2007; Hall and Peters, 2008; Kleindorfer et al., 2013b; Cain et al., 2015; Dowling and Webster, 2016). The *Malurus* genus remain an ideal model system to examine patterns of female song and singing behaviour and the evolution of song in both sexes because fairy-wrens (1) produce duets, choruses and solo song, (2) song mainly functions for territory defence, and (3) social and ecological selection pressures vary between species (Russell and Rowley, 1997; Greig et al., 2013). Comparative studies on fairy-wren species should be conducted to further explore the role of females in territory defence, and possible alternative functions of male and female song.

Fitness (survival value)

This thesis sheds light on the adaptive significance of male and female song by exploring the role of female song for pairing outcome (Chapter 4) and sex differences in costs of adult song (Chapter 5). We found that there was a positive association between the observed element repertoire size of females and the proportion of shared elements with the pair male in social pairs (Chapter 4). Yet we also found a significant negative correlation for song complexity within pairs, which contradicts current theory about mate choice in relation to song complexity. If chatter song functions for mate choice, then one would predict that males and females pair positively and assortatively for song complexity because it is a well-documented honest signal of individual quality (Buchanan et al., 2004). Instead, we found the opposite pattern. We argue that this pattern may have arisen from females with complex song pairing with males that share a higher proportion of shared elements for better-coordinated territorial defence, since territories are a valuable shared resource (Chapter 4). Thus, chatter song may be indirectly associated with mate choice as a result of females choosing mates for territory defence to increase their fitness. Mate choice experiments should be conducted to determine whether females with complex song prefer males with shared element types.

How male and female song is implicated in territory defence could be investigated to understand why female (and male) song is so complex in this species, and to provide greater insight into the relationship between female song and female competition. We found that male and female song rates were positively correlated and highest during the pre-breeding period, a time when pairs and territories are established, and fairy-wrens produce song in response to song playback (Chapter 5; also see Cooney and Cockburn, 1995; Kleindorfer et al., 2013b). In superb fairy-wrens and splendid fairy-wrens, both sexes respond first to the song playback of same-sex intruders (Kleindorfer et al., 2013b; Colombelli-Négrel, 2016). Therefore fairy-wrens use song to distinguish the sex of intruder birds (Kleindorfer et al., 2013b). Yet it remains to be studied whether song complexity and song rate are important signals of competitive ability, and correlate with measures of fitness in this system, as has been proposed in other species with female song (Brunton et al., 2016).

My thesis also identified sex differences in costs of adult singing behaviour whereby females that sang inside the nest at a higher rate had higher nest predation than males that sang at a higher rate close to the nest (Chapter 5). Thus, females incur high fitness costs for singing inside the nest. Why then, did 50% of females show this behaviour? Female superb fairy-wrens only sang in the nest in response to their social mate's song (Chapter 5). We propose that chatter song is an important form of social communication and that it may also function for within-pair communication in the form of coordination of breeding activities and nest defence. Song element sharing within pairs may facilitate within-pair communication and strengthen pair bonds between males and females (Hile et al., 2005).

Females with complex song that paired with males for shared element types may receive additional benefits in the form of increased transmission of element types shared with pair male to offspring, and reduced individual song output. Perhaps females with complex song sing less frequently than females with simple song because the social mate sings the same element types. If one function of shared element repertoire is to enhance exposure of female element types and lower song output, then females may receive indirect benefits by choosing shared vocal element repertoire for offspring fitness and direct benefits by lower song rate and hence lower predation risk. To test this, female song rate should be lower in pairs with high element sharing, yet offspring vocal copy accuracy should also be higher. Both of these ideas remain to be tested.

Acknowledged limitations of thesis

The conclusions of my thesis are tempered by a few limitations, which I summarise here. Not all individuals per population were sampled, and the high rates of nest predation and fledgling predation resulted in small samples sizes. These are key constraints of field studies and limit the confidence of certain analyses (Chapter 2 – 4). I did not assess whether neighbours were also vocal tutors for fledged superb fairy-wrens. Rather, I focused on song learning and production within groups, since fledglings predominantly interacted with social mothers, fathers, and helpers during the observation periods. Nonetheless, neighbours could be potential vocal tutors, but it remains to be tested whether fledglings learn from individuals outside of the natal group. Furthermore, it has not yet been examined whether females share more element types with their social mates than with neighbours. Colombelli-Négrel (2016) found song element sharing with neighbours in splendid fairy-wrens and variegated fairy-wrens, but only splendid fairy-wren females shared more elements with their mate than neighbours. Another limitation of my thesis is the use of ‘observed repertoire size’ rather than a rigorous estimate of ‘total element repertoire size’ for individual birds. It is likely that ‘observed element repertoire size’ underestimated ‘total element repertoire size’. I did not have sufficient numbers of song recordings to confidently measure total element repertoire size (a challenge in the wild when a family of birds will not all sing at the same time) and therefore I did not attempt to suggest an accurate measure of total repertoire size, but used observed repertoire size as a proxy. Given that observed repertoire size did not change significantly across years in the colour-banded birds I recorded, there is evidence that observed repertoire size was consistent. These limitations have been acknowledged in each chapter and the findings have been interpreted with caution.

Conclusion: giving females (songbirds) a voice

Gender issues in society and science are challenging across many levels and provide opportunity for insight (Gowaty, 1997; 2003; Hrdy, 1997). My thesis adds to a growing body of work that expands the theoretical understanding of female song (Langmore, 1998; Riebel, 2003; 2016; Garamszegi et al., 2006; Price et al., 2009; Tobias et al., 2012; Odom et al., 2014; Lobato et al., 2015; Price, 2015; Brunton et al., 2016) in a system in which most theory has been developed to explain male song. In general, the outcomes of this body of work are expected to impact both the social and natural sciences given their mutually reciprocal influence on the development of human thought (e.g. Medicus, 2015). As a final note, this journey has been particularly illuminating for me as a female scientist, and for my colleagues as we navigate the course of gender inclusion.

Appendix

Appendix 1

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When subspecies matter: resident Superb Fairy-wrens (*Malurus cyaneus*) distinguish the sex and subspecies of intruding birds

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Abstract. The widely accepted functions of complex bird song – to defend a territory or attract a mate, or both – have generally been tested in northern hemisphere species in which males produce the song and females choose the singer. In our study species, the Superb Fairy-wren (*Malurus cyaneus*), both males and females sing a solo song throughout the year. We compare the chatter song in males and females of two genetically distinct subspecies, and test if resident birds respond to the sex and subspecies of the intruder song. Compared with island birds (*M. c. ashbyi*), mainland Superb Fairy-wrens (*M. c. leggei*) produced songs with lower frequency and fewer elements. Compared with females, males produced longer songs with more elements. Resident birds showed acoustical discrimination for the sex and subspecies of the intruder bird. The response of resident pairs was positively correlated, but each sex showed a solo response. Resident males were the first to respond to male intruders, and resident females were the first to respond to female intruders. Fairy-wrens had the strongest response towards (1) intruders of the same subspecies and (2) male intruders. The finding of signal divergence and acoustical discrimination in males and females makes this a model system to test the mechanism of reproductive isolation when both sexes sing.

Additional keywords: geographical variation, mating signal divergence, pre-mating barrier, reproductive isolation, song dialect, species recognition.

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Introduction

Divergence in mating signals is an important evolutionary process that may lead to reproductive isolation and speciation (Slabbekoorn and Smith 2002; Price 2008; Irwin *et al*. 2008). The behavioural mechanisms that create pre-mating barriers to reproduction are particularly important for the initial process of divergence (Price 2008; van Doorn *et al*. 2009). Songbirds are good model systems to examine discrimination of mating signals because song is widely used for species recognition and mate-choice. The functions of song discrimination include the avoidance of costly hybrid mating (via species recognition) and the identification of quality mates (via mate-choice) (Petrinovich and Patterson 1981; Grant and Grant 1996; Ratcliffe and Otter 1996; Price 2008; Irwin *et al*. 2008).

Geographical song variation can arise for different reasons, including variation in the genetic predisposition for song learning, variation in song tutors for learned song characteristics, and patterns of morphological constraints on song production (reviewed in Marler and Slabbekoorn 2004; Podos and Nowicki 2004; Price 2008; Fehér *et al*. 2009). Song-variants can restrict gene flow if females prefer a particular song-variant, thus reinforcing genetic differentiation between populations (Baker 1983). There is evidence that geographically distinct songs promote assortative pairing for song-type in at least some species (Baker 1983; Blackmore 2002). There is also evidence that songbirds are more responsive to their local song dialects, when present, than to distantly related dialects, or to songs of other species (Petrinovich and Patterson 1981; Baker 1983; Podos 2007, 2010; Derryberry 2011).

Here we test if song differs between two subspecies of the Superb Fairy-wren (*Malurus cyaneus*) in South Australia (SA): the Kangaroo Island subspecies (*M. c. ashbyi*) and the subspecies of the adjacent mainland of SA on the Fleurieu Peninsula (*M. c. leggei*). The subspecies have been geographically separated by a sea straight for *c*. 9000 years, following a rise in sea level at that time (Schodde 1982; Belperio and Flint 1999; Paton *et al*. 2002). We have previously shown that each subspecies represents a genetically distinct population with little gene flow between them (Dudaniec *et al*. 2011); we also showed that each subspecies differs in morphology and foraging behaviour

(Schlotfeldt and Kleindorfer 2006). Compared with mainland birds, island birds of both sexes: (1) occupied a wider niche breadth; (2) were significantly larger in body size and (3) had narrower bills (there was no difference in bill-length) (Schlotfeldt and Kleindorfer 2006; Dudaniec *et al*. 2011; see also Myers *et al*. 2010). The Superb Fairy-wren is a non-duetting species in which both males and females sing a solo chatter song throughout the year to defend their permanent territories (Cooney and Cockburn 1995; Rowley and Russell 1997; Dalziell and Cockburn 2008). They are, therefore, a model system for testing the response of resident males and females to the singing behaviour of male and female intruders.

Most studies of song discrimination have been on species in which only males sing (Garamszegi *et al*. 2006). The tropics have a high proportion of duetting species, and there are a growing number of studies on the function of duets (e.g. Thorpe *et al*. 1972; Grafe and Bitz 2004; Mennill and Vehrencamp 2008; Hall 2009; Dowling and Webster 2013). The southern hemisphere has a high proportion of species with solo male and female song (Langmore 1998; Slater and Mann 2004; Greig and Pruett-Jones 2008; Greig *et al*. 2012), although song discrimination by both sexes in these systems is largely unstudied. Song discrimination is usually tested by broadcasting the song of a male bird from speakers in the territory of a focal pair and measuring the response of either or both sexes (Searcy and Marler 1981; Dabelsteen 1988; Searcy and Brenowitz 1988; Searcy and Yasukawa 1996; Derryberry 2007; Podos 2010). There are many challenges in developing experimental designs to test song discrimination, including differences between sexes in response selectivity, hormonal manipulation of study subjects and field *v.* laboratory conditions (Searcy and Brenowitz 1988). Despite these challenges, researchers have accumulated a wealth of knowledge about differences between sexes in the behavioural response of resident birds to playback of male song (Beletsky *et al*. 1980; Baker *et al*. 1981; Baker 1983; Searcy and Brenowitz 1988; Clayton and Pröve 1989). In general, males respond with aggressive territorial defence to playback of male intruders whereas female response is measured as copulation solicitation display, nest-building activity and date of laying (reviewed in Nowicki and Searcy 2005; Danner *et al*. 2011). We will test song discrimination in a system where both males and females use chatter song to defend the permanent territory (Cooney and Cockburn 1995).

We have two main aims: (1) to determine whether chatter song differs between male and female Superb Fairy-wrens in the two subspecies and (2) to determine whether resident Fairy-wrens acoustically discriminate between intruder Fairy-wrens on the basis of their subspecies and sex. We broadcast chatter song of male and female fairy-wrens from speakers in permanent territories and measure the response of the resident males and females. We also use the song from a congener, the Variegated Fairy-wren (*Malurus lamberti*), as a control. For the song structure we predict: (1) females will have more unique elements than males because males are philopatric and females disperse from their natal territory (Mulder 1995; Double and Cockburn 2000; Schlotfeldt 2010); (2) the subspecies will differ in song because they have been separated for *c*. 9000 years with little dispersal (Dudaniec *et al*. 2011) and (3) males will have more complex songs (number of elements per song) than females because extra-pair paternity is predicted by the intensity and complexity of male song (Cockburn *et al*. 2009). For the playback experiments, we predict a stronger response to intruders of the same subspecies, because local Fairy-wrens pose a greater risk of mate competition and territory theft (Petrinovich and Patterson 1981; Baker 1983; Podos 2007, 2010).

Materials and methods

Study species

The Superb Fairy-wren is a small insectivorous songbird endemic to south-eastern Australia (Rowley and Russell 1997). Males are philopatric whereas females disperse 1–10 km from their natal territory before forming a long-term pair that defends a shared territory for 5–10 years (e.g. Double and Cockburn 2000). Superb Fairy-wrens have a cooperative mating system that is notorious for many extra-pair copulations; ~70–95% of nests have at least one extra-pair young (Mulder *et al*. 1994; Double and Cockburn 2003; Cockburn *et al*. 2013). In our study population in SA, there were virtually no auxiliary males in any territory but a high percentage (83%) of nests nevertheless contained extra-pair young (Colombelli-Négrel *et al*. 2009). Males and females can easily be distinguished in the field: females have brown plumage and orange-red lores, eye-ring and bill; breeding males are strikingly different, with conspicuous blue cap, ear-coverts and mantle, a blue-black tail and a black bill; non-breeding males have brown, female-like plumage but with black lores and bill and the blue-black tail of the breeding plumage (Rowley and Russell 1997; Peters *et al*. 2013).

The species is known for its complex vocalisations, with eight types described: Type I song (chatter song), Type II song (trill song), Type III song (alarm song), alarm call, incubation call, brooding purr, feeding-young call and contact call (reviewed in Colombelli-Négrel 2008; Colombelli-Négrel *et al*. 2011, 2012). Here we focus on the Type I or chatter song because it is the most common vocalisation sung by both males and females, it is used by both sexes for territorial defence, and is used by males for attracting extra-pair copulations (Langmore and Mulder 1992; Cooney and Cockburn 1995; Dalziell and Cockburn 2008; Cockburn *et al*. 2009). Evidence for a territorial defence function of chatter song is: (1) rates of chatter song increase at the onset of the breeding season after several months of communal foraging; (2) birds in newly established territories have higher rate of chatter song and (3) females are more likely to produce chatter song in response to intruding neighbours and strangers than to their mates (Cooney and Cockburn 1995). Evidence for a mate-attraction function of chatter song includes: (1) males sing chatter song in a dawn chorus; (2) males in the dawn chorus are visited by females seeking extra-pair copulations and (3) males that produce more chatter song have more extra-pair copulations (Dalziell and Cockburn 2008). The role of female preference for male chatter song and reproductive success has not yet been studied, although we predict assortative pairing for this trait given that both males and females produce the chatter song.

Subspecies and study site

We collected song recordings in six study areas in SA. Recordings were made at three mainland sites on the Fleurieu Peninsula (*M. c. leggei*): Scott Creek Conservation Park (CP)

(35°05′S, 138°41′E), Scott CP (35°24′S, 138°44′E) and Newland Head CP (35°37′S, 138°29′E); and three island sites on Kangaroo Island (*M. c. ashbyi*): Flinders Chase National Park (NP) (35°54′S, 136°47′E), Pelican Lagoon CP (35°48′S, 137°48′E) and Vivonne Bay CP (36°00′S, 137°09′E). The study sites were selected to build on long-term data collected by the BirdLab at Flinders University on Fairy-wren population genetic structure (Schlotfeldt 2010; Dudaniec *et al*. 2011; Kleindorfer *et al*. 2013).

Song recordings

Overall, we recorded the chatter song of 104 males and 92 females (67 colour-banded Fairy-wrens and 129 birds sampled along transects; recordings of unbanded birds were separated by at least 200 m). On the mainland (*M. c. leggei*), sample size per study site was 14 males and 12 females at Scott Creek CP, 22 males and 15 females at Scott CP and 17 males and 17 females at Newland Head CP. On Kangaroo Island (*M. c. ashbyi*) sample size was 14 males and 18 females at Flinders Chase NP, 18 males and 13 females at Pelican Lagoon CP and 19 males and 17 females at Vivonne Bay CP. In all cases, we used only the male or female from each territory to assure independence of the song recordings for analysis. We recorded a mean of 6.1 songs per Fairy-wren (s.e. 1.1) at a distance of 5–20 m. Recordings were made with a Telinga Twin Science parabolic microphone (Telinga Microphones, Tobo, Sweden), connected to a portable Sound Devices 702 digital audio recorder (Sound Devices LCC, USA). All sound files were recorded as broadcast wave files (24 bit 48 kHz).

Acoustical analysis

We transcribed all sound files to an Apple MacPro (Apple Corporation, USA) for editing with Amadeus Pro 1.3.2 (HairerSoft, Switzerland) and analysis with Raven Pro 1.4 (Charif *et al*. 2008). The chatter songs used for playback were high-pass filtered at 1 kHz to reduce background noise.

Spectrograms were produced using the Hann algorithm in Raven Pro 1.4 (filter bandwidth 124 Hz, 512 samples, time-grid overlap 50%, grid-resolution 5.8 ms). For each song, we measured the minimum and maximum frequency (Hz), frequency bandwidth (Hz), duration (s), the total number of elements per song and the number of different elements per song. Using classifications developed by Langmore and Mulder (1992), Blackmore (2002), Dalziell and Cockburn (2008) and Colombelli-Négrel *et al*. (2011) and comparing existing classifications of elements to our own songs, we identified nine elements in our samples, which we refer to as elements A, F, O, R, T, U, V, W and Y (Fig. 1). The total number of elements per song, and the number of different elements per song were used in statistical analyses.

Playback stimuli

We used three types of playback stimuli: (1) same subspecies (island song played to island Fairy-wrens or mainland song played to mainland Fairy-wrens); (2) different subspecies (mainland song played to island Fairy-wrens and vice versa) and (3) control song of Variegated Fairy-wren, a congener that occurs on both the island and the mainland. We used a different song for each playback trial: 99 songs from the island subspecies (*M. c. ashbyi*), 97 songs from the mainland subspecies (*M. c. leggei*), and five control songs from five male Variegated Fairy-wrens (from Calperum Station, near Renmark, SA). We used Amadeus Pro (HairerSoft, Kenilworth, UK) to make playback stimuli that repeated every 10 s. Stimuli were played on a FOXPRO Scorpion X1-B (FOXPRO Inc., Lewistown, PA, USA) that could be remotely controlled when the focal Fairy-wrens were nearby. There were 178 playback trials in which each territory was tested only once with one of the five types of stimuli (same subspecies male, same subspecies female, different subspecies male, different subspecies female, control). To ensure social independence of the singer for the recording we never used a recording from the focal pair in playback experiments.

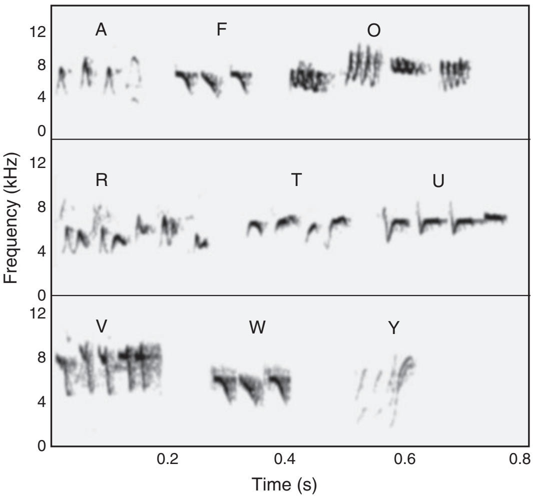

Fig. 1. Spectrograms of the different elements identified in the chatter song of Superb Fairy-wrens (*Malurus cyaneus*) from Kangaroo Island (*M. c. ashbyi*) and the adjacent mainland on the Fleurieu Peninsula (*M. c. leggei*). The elements are: A, mainland female; F, island male; O, mainland male; R, island female; T, mainland male; U, island male; V, mainland male; W, island male; and Y, mainland male.

Playback experiments

We colour-banded 354 Fairy-wrens and recorded response to playback of 107 colour-banded and 71 unbanded Fairy-wrens. The playback experiments were done in September and October in 2010 and 2011 at two study sites for each subspecies: Scott Creek CP and Sandy Creek CP on the mainland (*M. c. leggei*) and Flinders Chase NP and Pelican Lagoon CP on Kangaroo Island (*M. c. ashbyi*). The sample size and origin of playback stimuli were as follows: 91 mainland experiments (17 different subspecies male, 19 different subspecies female, 21 same subspecies male, 14 same subspecies female, 20 controls) and 87 island experiments (16 same subspecies male, 19 same subspecies female, 15 different subspecies male, 17 different subspecies female, 20 controls). For the playback trials, we used a similar number of playback stimuli from the three study sites for each subspecies to avoid a stronger response by local birds to playback of local song, for example. We entered study site of the playback origin as a covariate in statistical analyses.

Recordings were played back to the male and female of pairs together, but the playback stimulus consisted of only one simulated intruder (either a male or female intruder of a particular subspecies). Each resident pair received only one playback stimulus (one of the following five possibilities: male same subspecies, female same subspecies, male different subspecies, female different subspecies, control). Once we located a test group (based on our long-term monitoring of territories; Colombelli-Négrel 2008), we placed the FOXPRO Scorpion speaker at the base of a shrub in the territory, ~20 m from three observers (see below). The playback trial began within 1 min of placing the speaker in the territory to ensure the focal Fairy-wrens were present and behaving normally. The playback was started using remote control of the FOXPRO speaker. The playback trial consisted of 3 min of silence and pre-playback observations (pre-trial), followed by 3 min of stimulus and observations during the playback of the intruder song (trial). For the 6-min period of observation for each experiment, three observers were hidden in the vegetation: one focussed on the behaviour of the resident breeding male, one focussed on the behaviour of the resident breeding female, the third focussed on behaviour by other species or neighbouring birds. We recorded the following behaviour for the pre-trial and trial: (1) number of male and female chatter songs per minute given within 20 m of the playback speaker; (2) number of movements over the playback speaker (passes) by the resident male and female combined and, separately for the male and female, (3) latency of response (s) within 10 m of the playback speaker; (4) closest approach the playback speaker (m) and (5) the first sex to respond (shortest latency). Fairy-wrens were observed to resume normal activity within 5 min of the completion of each playback trial.

Statistical analyses

We used PASW Statistics for statistical analyses (PASW version 18.0 for Windows, SPSS Inc., Chicago, IL). For the analyses of song characteristics, we determined the mean per bird for each song variable: minimum frequency (Hz), maximum frequency (Hz), frequency bandwidth (Hz), duration (s) and number of elements per song. We used two separate multivariate analyses of variance (MANOVA) to test for song characteristics and the proportion of elements; in both models, we tested for effects of subspecies and sex on the dependent variables. To analyse the proportion of elements per song, we square-root transformed the values per element. There were no significant differences in the pre-trial behaviours between Fairy-wren territories (all $P > 0.07$; data available from the authors upon request). Therefore, we exclude pre-trial behaviour from further analysis and focus on trial response. We used regression analysis to test if the male and female response in each territory was correlated. We analyse the combined response of the pair to intruder subspecies and intruder sex using MANOVA, with all response variables (chatter song, latency to respond, closest approach, number of passes over the speaker) as the dependent variables. In addition, we were specifically interested in the response patterns of males and females. We used principal components analysis (PCA) to create single derived-response variables for each sex separately. The PCA variable male response had an eigenvalue of 2.32, which explained 58% of the variance; the PCA variable female response had an eigenvalue of 2.48, which explained 62% of the variance (see Table 1 for factor loadings). Both PCA variables had positive factor loadings for number of chatter songs and number of passes over the playback speaker, and negative factor loadings for latency to respond and closest approach to the playback speaker. Therefore, high PCA scores indicate a strong response (many vocalisations, many passes over the speaker, short time to respond, and close approach distance). We used MANOVA to test for the response intensity of males and females in relation to intruder subspecies and sex, and entered geographical origin of the playback (stimulus origin) and the identification of the pair as covariates.

2098

2099

Results

Chatter song

The characteristics of the chatter song differed significantly for each subspecies and sex (Tables 2, 3). There was no effect of the covariate study site. MANOVA showed there was a significant

Table 1. The factor loadings from Principal Components Analysis (PCA) of response variables of resident Superb Fairy-wrens to experimental playback of song of intruder Fairy-wrens

A high PCA score indicates a strong response (many vocalisations, short time to respond, close approach to the speaker, many passes over the speaker)

Response variables	PCA	
	Male response	Female response
Male chatter song	0.67	
Male latency to respond	–0.79	
Male closest approach	–0.83	
Male passes over speaker	0.75	
Female chatter song		0.80
Female latency to respond		–0.76
Female closest approach		–0.82
Female passes over speaker		0.77

Table 2. Song characteristics (mean ± s.e.) of male and female Superb Fairy-wrens: (*a*) *M. c. ashbyi* on Kangaroo Island and (*b*) *M. c. leggei* on the mainland Fleurieu Peninsula

	Male ($n = 51$)	Female ($n = 48$)
(*a*) Kangaroo Island (*M. c. ashbyi*)		
Minimum frequency (Hz)	3 113.9 ± 63.6	3 391.0 ± 67.6
Maximum frequency (Hz)	10 993.5 ± 88.0	10 904.1 ± 99.0
Frequency bandwidth (Hz)	2 759.2 ± 66.1	2 725.4 ± 79.2
Song duration (s)	3.1 ± 0.2	2.8 ± 0.2
Number of elements per song	42.8 ± 3.0	34.3 ± 1.9
Number of different elements	5.5 ± 0.2	4.7 ± 0.1
	Male ($n = 53$)	Female ($n = 44$)
(*b*) Mainland (*M. c. leggei*)		
Minimum frequency (Hz)	2 827.2 ± 85.26	2 867.9 ± 76.3
Maximum frequency (Hz)	10 558.0 ± 99.6	10 488.0 ± 93.5
Frequency bandwidth (Hz)	3 038.5 ± 94.3	2 871.6 ± 84.2
Song duration (s)	2.9 ± 0.1	2.6 ± 0.1
Number of elements per song	42.5 ± 3.2	37.7 ± 2.4
Number of different elements	4.5 ± 0.2	4.5 ± 0.2

effect of subspecies on minimum frequency and maximum frequency, and an effect of sex on minimum frequency, song duration and the number of elements per song (Table 3). There was a significant effect of the interaction term subspecies × sex for number of elements: island males produced songs with a greater number of different elements than mainland males (Table 3). Mainland Fairy-wrens produced songs with lower minimum frequency, lower maximum frequency, and a narrower frequency bandwidth than island Fairy-wrens (Table 2). Compared with females, males generally produced longer songs with a lower minimum frequency and a higher number of elements (Table 2).

Table 3. Results of multivariate analyses of variance (MANOVA) of song characteristics across study sites and sex for Kangaroo Island (*M. c. ashbyi*) and mainland (*M. c. leggei*) subspecies of Superb Fairy-wren ($n = 196$ songs)

Variables that were significantly different ($P < 0.05$) are marked in bold. Study site was a covariate

	d.f.	*F*	*P*
Study site			
Minimum frequency (Hz)	5	0.6	0.438
Maximum frequency (Hz)	5	1.2	0.269
Frequency bandwidth (Hz)	5	0.7	0.406
Song duration (s)	5	0.3	0.585
Number of elements per song	5	0.4	0.504
Number of different elements	5	0.2	0.644
Subspecies			
Minimum frequency (Hz)	1	9.4	**0.003**
Maximum frequency (Hz)	1	7.1	**0.008**
Frequency bandwidth (Hz)	1	4.0	**0.048**
Song duration (s)	1	0.03	0.866
Number of elements per song	1	0.8	0.376
Number of different elements	1	0.7	0.407
Sex			
Minimum frequency (Hz)	1	4.4	**0.037**
Maximum frequency (Hz)	1	0.9	0.347
Frequency bandwidth (Hz)	1	1.5	0.226
Song duration (s)	1	4.4	**0.037**
Number of elements per song	1	5.1	**0.025**
Number of different elements	1	2.3	0.130
Subspecies × Sex			
Minimum frequency (Hz)	1	1.8	0.186
Maximum frequency (Hz)	1	0.03	0.857
Frequency bandwidth (Hz)	1	0.002	0.96
Song duration (s)	1	0.4	0.517
Number of elements per song	1	0.6	0.428
Number of different elements	1	9.24	**0.003**

Song-elements between subspecies

Fig. 1 shows spectrograms of the nine elements we identified in male and female chatter song in our study populations; all elements occurred in both subspecies. We found significant differences between subspecies in the number of elements F, T, V, W (Table 4, Fig. 2) and differences between males and females in the number of elements T and Y (Table 4, Fig. 2).

Playback experiment

There was a positive correlation between the response of the resident male and resident female Fairy-wren (PCA factor scores) to intruder males and females of the same subspecies (male intruder: $r = 0.773$, $t = 6.784$, $P < 0.001$; female intruder: $r = 0.353$, $t = 2.04$, $P = 0.05$) and different subspecies (male intruder: $r = 0.461$, $t = 2.651$, $P = 0.013$; female intruder: $r = 0.407$, $t = 2.639$, $P = 0.012$) (Fig. 3). However, there was no significant correlation between the response of the resident male and resident female to the control stimulus ($r = 0.223$ $t = 1.584$, $P = 0.120$; Fig. 3).

Given the positive correlation between response of the resident male and resident female, we tested the overall response of the pair to intruder subspecies and intruder sex, with the covariate stimulus origin (study site of the song for the playback). The dependent variables were chatter song, latency to respond, closest approach, and number of passes over the speaker for the resident pair. There was a significant difference in response intensity for intruder subspecies and intruder sex (MANOVA: Intruder subspecies: chatter song $F_{1,131} = 12.71$, $P = 0.001$; latency $F_{1,131} = 5.78$, $P = 0.018$; closest approach $_{1,131} = 5.74$, $P = 0.018$; number of passes $F_{1,131} = 5.13$, $P = 0.025$. Intruder sex: chatter song $F_{1,131} = 17.93$, $P = 0.001$; latency $F_{1,131} = 3.54$, $P = 0.062$; closest approach $F_{1,131} = 5.18$, $P = 0.025$; number of passes $F_{1,131} = 7.21$, $P = 0.008$) (Fig. 4). The interaction term

Table 4. The mean number of chatter song elements in songs of the two subspecies of Superb Fairy-wren ($n = 196$ songs)

Figures under each subspecies are means ± s.e., with percentage of songs that were made up of each element in parentheses. Statistical results are shown for the comparison of the two main effects (sex and subspecies); the interaction term sex × subspecies was not significant (MANOVA). Variables that were significantly different ($P < 0.05$) are marked in bold

Song element	Kangaroo Island (*M. c. ashbyi*) Males	Kangaroo Island (*M. c. ashbyi*) Females	Mainland (*M. c. leggei*) Males	Mainland (*M. c. leggei*) Females	Sex *F*	Sex *P*	Subspecies *F*	Subspecies *P*
A	3.7 ± 0.5 (8%)	2.6 ± 0.5 (8%)	3.7 ± 0.6 (8%)	5.1 ± 0.5 (13%)	0.03	0.867	2.68	0.104
F	4.1 ± 0.5 (8%)	3.9 ± 0.5 (11%)	2.7 ± 0.5 (6%)	2.4 ± 0.4 (5%)	0.20	0.657	**5.26**	**0.023**
O	11.5 ± 0.7 (28%)	11.9 ± 0.7 (34%)	13.8 ± 0.8 (33%)	11.8 ± 0.7 (32%)	0.58	0.447	1.04	0.310
R	4.9 ± 0.5 (11%)	4.0 ± 0.5 (12%	6.3 ± 0.7 (15%)	5.6 ± 0.6 (14%)	0.56	0.455	2.32	0.129
T	9.4 ± 0.7 (22%)	6.5 ± 0.7 (19%)	11.0 ± 0.8 (26%)	9.4 ± 0.6 (26%)	**4.90**	**0.028**	**4.82**	**0.030**
U	4.5 ± 0.5 (10%)	3.3 ± 0.5 (9%)	3.7 ± 0.6 (9%)	2.3 ± 0.4 (6%)	3.10	0.080	1.44	0.233
V	1.8 ± 0.3 (4%)	0.7 ± 0.3 (2%)	0.3 ± 0.3 (1%)	0.2 ± 0.2 (1%)	3.34	0.070	**9.71**	**0.002**
W	2.5 ± 0.4 (6%)	1.5 ± 0.4 (5%)	0.6 ± 0.4 (2%)	0.7 ± 0.3 (2%)	1.02	0.315	**9.72**	**0.002**
Y	0.7 ± 0.2 (2%)	0 ± 0 (0%)	0.6 ± 0.2 (1%)	0.3 ± 0.1 (1%)	**4.42**	**0.037**	0.14	0.706

2100

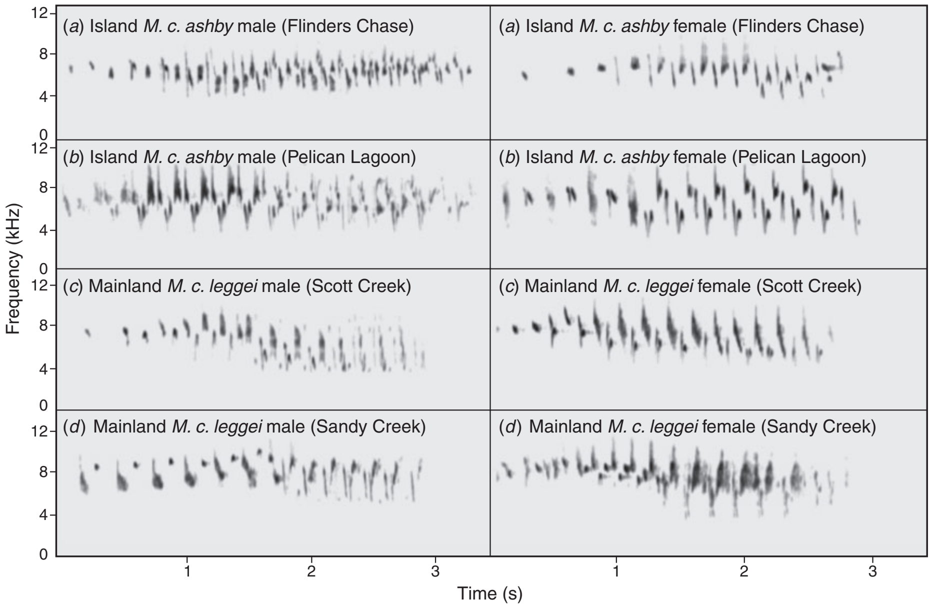

Fig. 2. Spectrograms of chatter song of: (*a–b*) the Kangaroo Island subspecies (*M. c. ahbyi*): (*a*) male and female from Flinders Chase; (*b*) male and female from Pelican Lagoon; and (*c–d*) the mainland subspecies (*M. c. leggei*) from the Fleurieu Peninsula: (*c*) male and female from Scott Creek; (*d*) male and female from Sandy Creek. Note that each song is composed of repeats of ~5 elements, with a total of ~40 elements per song (see Table 2 for details of song characteristics).

intruder subspecies × intruder sex, however, was not significant (all $P > 0.07$) nor was the covariate stimulus origin (all $P > 0.1$).

Despite the correlated response in each territory, we were interested in the patterns of male and female response in each territory. Analysing the derived principal components response scores for resident males and females, we found the same pattern as the response of the pair: resident male and female Fairy-wrens had a significantly higher response to intruder Fairy-wrens of the same subspecies, and to male intruder Fairy-wrens (Tables 5, 6; Figs 5, 6). The interaction term intruder subspecies intruder sex was not significant.

Examining the responses of males and females separately, we found one significant difference between sexes. Comparing the same subspecies, resident male Fairy-wrens were more often (77%) the first sex to respond to a male intruder (24 of 31 trials) and female Fairy-wrens were more often (61%) the first to respond to female intruders (19 of 31 trials) ($\chi^2 = 9.54$, d.f. = 1, 62, $P = 0.002$). But when intruder Fairy-wrens were a different subspecies, there was no difference in the likelihood that the resident male or female Fairy-wren would respond first ($\chi^2 = 0.410$, d.f. = 1, 59, $P = 0.522$).

Discussion

The main findings of this study are that: (1) the chatter song differed significantly between genetically distinct subspecies of Superb Fairy-wren in SA; (2) the chatter songs of males and females were significantly different, irrespective of subspecies; and (3) resident Superb Fairy-wrens were able to discriminate acoustically between intruder songs on the basis of both subspecies and sex.

The observed divergence in song in the two allopatric subspecies *M. c. ashbyi* (Kangaroo Island) and *M. c. leggei* (mainland SA) is probably a result of their separation for *c.* 9000 years. The two subspecies are genetically distinct, with little gene flow between Kangaroo Island and the mainland (Dudaniec *et al.* 2011). The findings of this study suggest that discrimination of chatter song could lead to accelerated divergence of the subspecies after secondary contact (Price 2008; Podos 2010).

Differences in song between subspecies

The songs of Kangaroo Island Superb Fairy-wrens (*M. c. ashbyi*) were more complex and had narrower bandwidth than the songs of mainland Fairy-wrens (*M. c. leggei*). Founder effects may explain divergent traits between geographical areas. Generally, the founding population has lower trait diversity (in this case, song) compared with the source population (MacArthur and Wilson 1967; Baker and Jenkins 1987; Lynch and Baker 1993). Several studies have found evidence for founder effects on geographical variation in song (Baker and Jenkins 1987; Lynch and Baker 1993; Baker *et al.* 2006), but the usual pattern is for reduced song complexity and fewer song-elements (Lynch and Baker 1993; Baker *et al.* 2006). In this study, island males had more complex songs with more song-elements than the mainland subspecies (Table 3). It is therefore unlikely that the observed differences are a result of founder effects from the colonist birds.

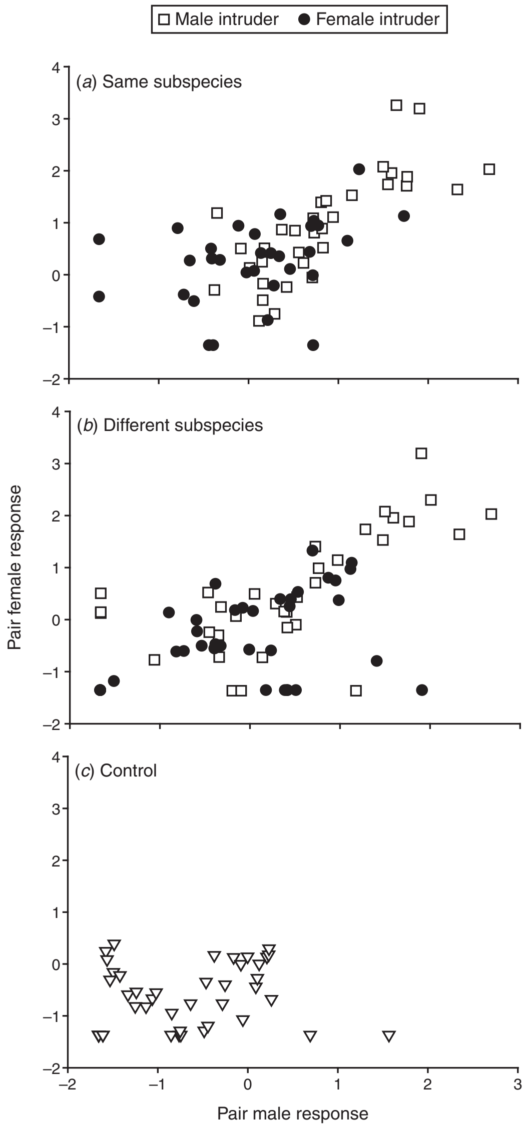

Fig. 3. The correlation between the response of the resident male and female (PCA factor scores) to intruder song of: (*a*) the same subspecies ($n = 35$ male intruders, 31 female intruders) or (*b*) a different subspecies ($n = 28$ male intruders, 37 female intruders). The response intensity of the resident birds was significantly correlated for male and female intruders of the same or different subspecies, but not for control playback ($n = 47$) (see Results).

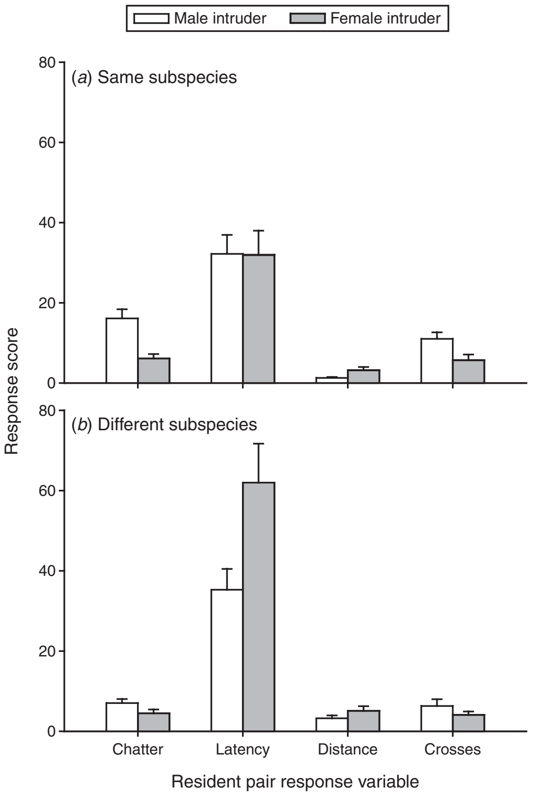

Fig. 4. The combined response of resident pairs of Superb Fairy-wrens to playback of a simulated intruder if the intruder was: (*a*) the same subspecies as the resident pair or (*b*) a different subspecies to the resident pair. Resident pairs sang more chatter song, had a shorter latency of response, a closer approach to the speaker, and flew over the playback speaker more often when the intruder was the same subspecies (see Results).

It is possible that the signal divergence was shaped by acoustical adaptation to vegetation characteristics of the environment (Morton 1975; Blumstein and Turner 2005; Boncoraglio and Saino 2007). Compared with the mainland, the vegetation of Kangaroo Island is dense (Schlotfeldt and Kleindorfer 2006), which would select for narrower frequency bandwidth, a result supported by data in Table 2.

The differences in song characteristics may be the result of morphological constraints on song production (Podos 2001; Huber and Podos 2006). Island and mainland birds did not differ in length of bill but island birds had larger body size (Dudaniec *et al*. 2011). Large body size is often associated with lower minimum frequency of song in interspecific comparisons (Greig *et al*. 2013). Contrary to the prediction, our intraspecific comparison found that the song of island birds had higher minimum frequency (Table 2).

Differences in song between the sexes

Male and female Fairy-wrens had different chatter songs. In both subspecies, chatter songs of males were longer, had lower minimum frequency, higher maximum frequency and broader frequency bandwidth than chatter songs of females. Further, males had more complex songs than females, given more elements

Table 5. The response (mean ± s.e.) of resident (*a*) male and (*b*) female Superb Fairy-wrens to playback of intruder song (same subspecies, different subspecies, control)

	Same Subspecies	Different subspecies	Control
(a) Resident male response			
Latency of response (s)	48.1 ± 5.5	69.5 ± 8.1	103.6 ± 9.7
Closest approach (m)	3.3 ± 0.6	5.5 ± 0.9	12.4 ± 1.1
Number of passes over the speaker	4.3 ± 0.6	3.1 ± 0.5	0.06 ± 0.04
Number of chatter songs	6.6 ± 0.9	3.2 ± 0.5	2.1 ± 0.8
(b) Resident female response			
Latency of response (s)	58.1 ± 7.6	86.5 ± 8.9	105.5 ± 10.7
Closest approach (m)	3.7 ± 0.6	7.6 ± 1.0	13.1 ± 1.1
Number of passes over the speaker	4.2 ± 0.7	2.0 ± 0.5	0.06 ± 0.04
Number of chatter songs	4.9 ± 0.6	2.4 ± 0.4	0.7 ± 0.2

Table 6. Results of multivariate analysis of variance (MANOVA) of the response of resident male and female Superb Fairy-wrens to playback of the song of an intruder Fairy-wren (178 independent trials)

We tested for the effects of intruder sex (male, female) and intruder subspecies (same subspecies, different subspecies) on the response of the resident male and female. Geographical origin of the playback and pair identification were covariates. Variables that were significantly different ($P < 0.05$) are marked in bold

Independent variables	Dependent variable	*F*	*P*
Intruder sex	Resident male response (PCA male)	**124.79**	**0.001**
	Resident female response (PCA female)	**90.27**	**0.001**
Intruder subspecies	Resident male response (PCA male)	**125.57**	**0.001**
	Resident female response (PCA female)	**86.82**	**0.001**
Intruder sex × Intruder subspecies	Resident male response (PCA male)	**9.73**	**0.002**
	Resident female response (PCA female)	0.48	0.490
Stimulus origin	Resident male response (PCA male)	2.17	0.143
	Resident female response (PCA female)	1.26	0.264
Pair identification	Resident male response (PCA male)	**111.84**	**0.001**
	Resident female response (PCA female)	**67.47**	**0.001**

per song, whereby island males had more element types than mainland males (Table 2). These differences in male song are consistent with sexual selection theory, and are in line with evidence of female preference for males that produce longer and more complex songs (Spencer *et al.* 2005; Dalziell and Cockburn 2008; Cockburn *et al.* 2009; reviewed in Byers and Kroodsma 2009). Sexual selection for greater duration and complexity of male song may be especially high in this species, given its notoriously high levels of extra-pair copulations (Double and Cockburn 2000; Cockburn *et al.* 2009; Colombelli-Négrel *et al.* 2009; Cockburn *et al.* 2013; Greig *et al.* 2013).

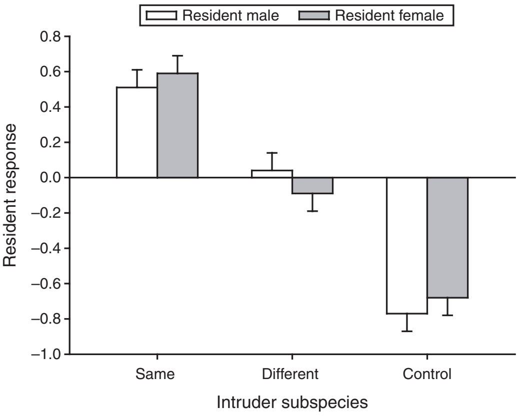

Fig. 5. The response of resident birds (shown as mean PCA factor scores ± s.e.) to playback song of a simulated intruder Fairy-wren in the territory in relation to intruder subspecies (178 playback trials: 66 same subspecies, 65 different subspecies, 47 control). High PCA values indicate a strong response (Table 1). Resident males and females had the strongest response to intruder birds of the same subspecies; the data were pooled across the sex of the simulated playback intruder.

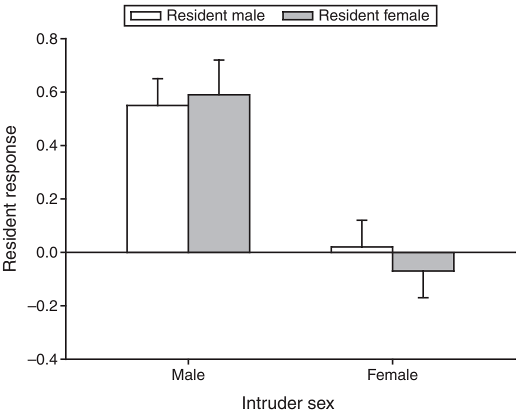

Fig. 6. The response of resident birds (shown as mean PCA factor scores ± s.e.) to playback song of a simulated intruder Fairy-wren in the territory in relation to intruder sex (178 playback trials: 66 same subspecies, 65 different subspecies, 47 control). High PCA values indicate a strong response (Table 1). Resident males and females had the strongest response to male intruders; the data were pooled across the subspecies of the simulated playback intruder.

Song discrimination by resident Fairy-wrens

The playback experiments showed that resident Fairy-wrens distinguished intruder Fairy-wrens acoustically on the basis of their subspecies and sex. The intensity of response by the resident male and female was positively correlated. This is similar to the results of a study that found a correlated duet response in pairs of male and female Purple-crowned Fairy-wrens (*M. coronatus*) (Hall and Peters 2008). However, in our study, the resident male and female had a correlated solo response to the simulated intruder. To more explicitly test the effect of the mate on response pattern, we need playback studies that restrain one member of the pair (e.g. Danner *et al*. 2011).

Resident females responded first to female intruders and resident males responded first to male intruders (see also Marshall-Ball and Slater 2004). Interestingly, the response pattern of the resident pair was correlated irrespective of the sex that was first to respond (see also Logue 2005; Gill 2012). This finding of sex-specific response to intruder identity but coordinated response of the resident pair in territorial defence provides support for the idea of social selection to defend shared resources (Langmore *et al*. 1996; Moravec *et al*. 2006; Tobias *et al*. 2012).

Most pairs responded most strongly to male intruders, despite the fact that females were the first sex to respond to female intruders and males were the first sex to respond to male intruders. We suspect this occurred because we did the playback trials during the fertile period, before laying, when the risk of extra-pair copulations was highest. But other explanations are possible. Male Superb Fairy-wrens are philopatric and an intruding male could be attempting a take-over of the territory (Pruett-Jones and Lewis 1990). Because our experimental intruder was simulated using the playback of song, we suspect that we would elicit more sex-specific behavioural responses using mounts or live birds to simulate intruders. Despite the limitations of the current study, we show that resident Fairy-wrens can distinguish the subspecies and sex of an intruding singer and alter their responses accordingly. This acoustical discrimination has the potential to restrict gene flow and increase reproductive isolation through pre-mating barriers.

In conclusion, there is compelling evidence that the island and mainland subspecies of Superb Fairy-wrens that we studied are two genetically distinct populations that are independently diverging; it is for this reason that both subspecies need to be considered in conservation planning (Ford *et al*. 2001; Dudaniec *et al*. 2011; Ford 2011; Szabo *et al*. 2011; Sunnucks 2011; Skroblin and Murphy 2013). Here we show that the process of divergence will be accelerated by signal discrimination for subspecies and sex. These findings provide a mechanism for limiting gene flow in addition to the prevailing argument that Fairy-wrens fail to disperse because their flight capacity is poor. The Maluridae are a model system for studies of sexual selection (Rowley and Russell 1997; Webster *et al*. 2007; Cockburn *et al*. 2008, 2009). Here we show their utility as a model system to study the processes that shape adaptive divergence in contiguous and allopatric populations (Endler 1977; Hendry *et al*. 2009; Galligan *et al*. 2012; Myers *et al*. 2012; Joseph and Edwards 2013).

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References

Baker, M. C. (1983). The behavioural response of female Nuttall's White-crowned Sparrows to male song of natal and alien dialects. *Behavioral Ecology and Sociobiology* **12**, 309–315. doi:10.1007/BF00302898

Baker, A. J., and Jenkins, P. F. (1987). Founder effect and cultural evolution of songs in an isolated population of Chaffinches, *Fringilla coelebs*, in the Chatham Islands. *Animal Behaviour* **35**, 1793–1803. doi:10.1016/S0003-3472(87)80072-6

Baker, M. C., Spitler-Nabors, K. J., and Bradley, D. C. (1981). Early experience determines song dialect responsiveness of female sparrows. *Science* **214**, 819–821. doi:10.1126/science.214.4522.819

Baker, M. C., Baker, M. S. A., and Tilghman, L. M. (2006). Differing effects of isolation on evolution of bird songs: examples from an island–mainland comparison of three species. *Biological Journal of the Linnean Society* **89**, 331–342. doi:10.1111/j.1095-8312.2006.00677.x

Beletsky, L. D., Chao, S., and Smith, D. G. (1980). An investigation of song-based species recognition in the Red-winged Blackbird (*Agelaius phoeniceus*). *Behaviour* **73**, 189–203. doi:10.1163/156853980X00221

Belperio, A. P., and Flint, R. B. (1999). Geomorphology and geology. In 'A Biological Survey of Kangaroo Island South Australia, in November 1989 and 1990'. (Eds A. C. Robinson and D. M. Armstrong.) pp. 19–31. (Heritage and Biodiversity Section, Department for Environment, Heritage and Aboriginal Affairs (SA): Adelaide.)

Blackmore, C. J. (2002). 'Male Song and the Dawn Chorus of the Superb Fairy-wren, *Malurus cyaneus*.' (Australian National University: Canberra.)

Blumstein, D. T., and Turner, A. C. (2005). Can the acoustic adaptation hypothesis predict the structure of Australian birdsong? *Acta Ethologica* **8**, 35–44. doi:10.1007/s10211-005-0107-7

Boncoraglio, G., and Saino, N. (2007). Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology* **21**, 134–142. doi:10.1111/j.1365-2435.2006.01207.x

Byers, B. E., and Kroodsma, D. E. (2009). Female choice and songbird song repertoires. *Animal Behaviour* **77**, 13–22. doi:10.1016/j.anbehav.2008.10.003

Charif, R. A., Waack, A. M., and Strickman, L. M. (2008). 'Raven Pro 1.3 User's Manual.' (Cornell Laboratory of Ornithology: Ithaca, NY.)

Clayton, N., and Pröve, E. (1989). Song discrimination in female Zebra Finches and Bengalese Finches. *Animal Behaviour* **38**, 352–354. doi:10.1016/S0003-3472(89)80096-X

Cockburn, A., Osmond, H. L., and Double, M. C. (2008). Swingin' in the rain: condition dependence and sexual selection in a capricious world. *Proceedings of the Royal Society of London –B. Biological Sciences* **275**, 605–612. doi:10.1098/rspb.2007.0916

Cockburn, A., Dalziell, A., Blackmore, C., Double, M. C., Kokko, H., Osmond, H. L., Beck, N. R., Head, M. L., and Wells, K. (2009). Superb Fairy-wren males aggregate into hidden leks to solicit extragroup fertilizations before dawn. *Behavioral Ecology* **20**, 501–510. doi:10.1093/beheco/arp024

Cockburn, A., Brouwer, L., Double, M. C., Margraf, N., and van de Pol, M. (2013). Evolutionary origins and persistence of infidelity in *Malurus*: the least faithful birds. *Emu* **113**(3), 208–217. doi:10.1071/MU12094

Colombelli-Négrel, D. (2008). The evolution of anti-predator responses and vocal communication in Superb Fairy-wrens (*Malurus cyaneus*). Ph. D. Thesis, Flinders University, Adelaide.

Colombelli-Négrel, D., Schlotfeldt, B. E., and Kleindorfer, S. (2009). High levels of extra-pair paternity in Superb Fairy-wrens in South Australia despite low frequency of auxiliary males. *Emu* **109**, 300–304. doi:10.1071/MU09035

Colombelli-Négrel, D., Robertson, J., and Kleindorfer, S. (2011). Risky revelations: Superb Fairy-wrens *Malurus cyaneus* respond more strongly to their mate's alarm song. *Journal of Ornithology* **152**, 127–135. doi:10.1007/s10336-010-0557-1

Colombelli-Négrel, D., Hauber, M. E., Robertson, J., Sulloway, F. J., Hoi, H., Griggio, M., and Kleindorfer, S. (2012). Embryonic learning of vocal passwords in Superb Fairy-wrens reveals intruder cuckoo nestlings. *Current Biology* **22**, 2155–2160. doi:10.1016/j.cub.2012.09.025

Cooney, R., and Cockburn, A. (1995). Territorial defence is the major function of female song in the Superb Fairy-wren, *Malurus cyaneus. Animal Behaviour* **49**, 1635–1647. doi:10.1016/0003-3472(95)90086-1

Dabelsteen, T. (1988). Do female Blackbirds, *Turdus merula*, decode song in the same way as males? *Animal Behaviour* **36**, 1858–1860. doi:10.1016/S0003-3472(88)80135-0

Dalziell, A. H., and Cockburn, A. (2008). Dawn song in Superb Fairy-wrens: a bird that seeks extrapair copulations during the dawn chorus. *Animal Behaviour* **75**, 489–500. doi:10.1016/j.anbehav.2007.05.014

Danner, J. E., Danner, R. M., Bonier, F., Martin, P. R., Small, T. W., and Moore, I. T. (2011). Female, but not male, tropical sparrows respond more strongly to the local song dialect: implications for population divergence. *American Naturalist* **178**, 53–63. doi:10.1086/660283

Derryberry, E. P. (2007). Evolution of bird song affects signal efficacy: an experimental test using historical and current signals. *Evolution* **61**, 1938–1945. doi:10.1111/j.1558-5646.2007.00154.x

Derryberry, E. P. (2011). Male response to historical and geographical variation in bird song. *Biology Letters* **7**, 57–59. doi:10.1098/rsbl.2010.0519

Double, M., and Cockburn, A. (2000). Pre-dawn infidelity: females control extra-pair mating in Superb Fairy-wrens. *Proceedings of the Royal Society of London – B. Biological Sciences* **267**, 465–470. doi:10.1098/rspb.2000.1023

Double, M., and Cockburn, A. (2003). Subordinate Superb Fairy-wrens (*Malurus cyaneus*) parasitize the reproductive success of attractive dominant males. *Proceedings of the Royal Society of London – B. Biological Sciences* **270**, 379–384. doi:10.1098/rspb.2002.2261

Dowling, J., and Webster, M. S. (2013). The form and function of vocal duets in Red-backed Fairy-wrens. *Emu* **113**(3), 282–293. doi:10.1071/MU12082

Dudaniec, R. Y., Schlotfeldt, B. E., Bertozzi, T., Donnellan, S. C., and Kleindorfer, S. (2011). Genetic and morphological divergence in island and mainland birds: informing conservation priorities. *Biological Conservation* **144**, 2902–2912. doi:10.1016/j.biocon.2011.08.007

Endler, J. A. (1977). 'Geographic Variation, Speciation and Clines.' (Princeton University Press: Princeton, NJ.)

Fehér, O., Wang, H., Saar, S., Mitra, P. P., and Tchernichovski, O. (2009). De novo establishment of wild-type song culture in the Zebra Finch. *Nature* **459**, 564–568. doi:10.1038/nature07994

Ford, H. A. (2011). The causes of decline of birds of eucalypt woodlands: advances in our knowledge over the last 10 years. *Emu* **111**, 1–9. doi:10.1071/MU09115

Ford, H. A., Barrett, G. W., Saunders, D. A., and Recher, H. F. (2001). Why have birds in the woodlands of southern Australia declined? *Biological Conservation* **97**, 71–88. doi:10.1016/S0006-3207(00)00101-4

Galligan, T. H., Donnellan, S., Sulloway, F. J., Fitch, A. J., Bertozzi, T., and Kleindorfer, S. (2012). Panmixia supports divergence with gene flow in Darwin's Small Ground Finch, *Geospiza fuliginosa*, on Santa Cruz, Galápagos Islands. *Molecular Ecology* **21**, 2106–2115. doi:10.1111/j.1365-294X.2012.05511.x

Garamszegi, L. Z., Pavlova, D. Z., Eens, M., and Møller, A. P. (2006). The evolution of song in female birds in Europe. *Behavioral Ecology* **18**, 86–96. doi:10.1093/beheco/arl047

Gill, S. A. (2012). Testing hypotheses for the evolution of long-term monogamy in Neotropical Buff-breasted Wrens (*Cantorchilus leucotis*). *Journal of Ornithology* **153**, 125–134. doi:10.1007/s10336-011-0804-0

Grafe, T. U., and Bitz, J. H. (2004). Functions of duetting in the tropical Boubou, *Laniarius aethiopicus*: territorial defence and mutual mate guarding. *Animal Behaviour* **68**, 193–201. doi:10.1016/j.anbehav.2003.11.003

Grant, B. R., and Grant, P. R. (1996). Cultural inheritance of song and its role in the evolution of Darwin's Finches. *Evolution* **50**, 2471–2487. doi:10.2307/2410714

Greig, E. I., and Pruett-Jones, S. (2008). Splendid songs: the vocal behaviour of Splendid Fairy-wrens (*Malurus splendens melanotus*). *Emu* **108**, 103–114. doi:10.1071/MU07044

Greig, E. I., Taft, B. N., and Pruett-Jones, S. (2012). Sons learn songs from their social fathers in a cooperatively breeding bird. *Proceedings of the Royal Society of London – B. Biological Sciences* **279**, 3154–3160. doi:10.1098/rspb.2011.2582

Greig, E. I., Price, J. J., and Pruett-Jones, S. (2013). Song evolution in Maluridae: influences of natural and sexual selection on acoustic structure. *Emu* **113**(3), 270–281. doi:10.1071/MU12078

Hall, M. L. (2009). A review of duetting in birds. *Advances in the Study of Behavior* **40**, 67–121. doi:10.1016/S0065-3454(09)40003-2

Hall, M. L., and Peters, A. (2008). Coordination between the sexes for territorial defence in a duetting fairy-wren. *Animal Behaviour* **76**, 65–73. doi:10.1016/j.anbehav.2008.01.010

Hendry, A. P., Huber, S. K., De León, L. F., Herrel, A., and Podos, J. (2009). Disruptive selection in a bimodal population of Darwin's finches. *Proceedings of the Royal Society of London – B. Biological Sciences* **276**, 753–759. doi:10.1098/rspb.2008.1321

Huber, S. K., and Podos, J. (2006). Beak morphology and song features covary in a population of Darwin's Finches (*Geospiza fortis*). *Biological Journal of the Linnean Society* **88**, 489–498. doi:10.1111/j.1095-8312.2006.00638.x

Irwin, D. E., Thimgan, M. P., and Irwin, J. H. (2008). Call divergence is correlated with geographic and genetic distance in Greenish Warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology* **21**, 435–448. doi:10.1111/j.1420-9101.2007.01499.x

Joseph, L., and Edwards, S. (2013). The Maluridae: inferring avian biology and evolutionary history from DNA sequences. *Emu* **113**(3), 195–207. doi:10.1071/MU12081

Kleindorfer, S., Evans, C., Colombelli-Négrel, D., Griggio, M., Hoi, H., and Robertson, J. (2013). Host response to cuckoo song is predicted by the future risk of brood parasitism. *Frontiers in Zoology* **10**, 30. doi:10.1186/1742-9994-10-30

Langmore, N. E. (1998). Functions of duet and solo songs of female birds. *Trends in Ecology & Evolution* **13**, 136–140. doi:10.1016/S0169-5347(97)01241-X

Langmore, N. E., and Mulder, R. A. (1992). Novel context for bird song: predator calls prompt male singing in the kleptogamous Superb Fairy Wren, *Malurus cyaneus. Ethology* **90**, 143–153. doi:10.1111/j.1439-0310.1992.tb00828.x

Langmore, N. E., Davies, N. B., Hatchwell, B. J., and Hartley, I. R. (1996). Female song attracts males in the Alpine Accentor *Prunella collaris. Proceedings of the Royal Society of London – B. Biological Sciences* **263**, 141–146. doi:10.1098/rspb.1996.0022

Logue, D. M. (2005). Cooperative defence in duet singing birds. *Cognition, Brain, Behavior* **9**, 497–510.

Lynch, A., and Baker, A. J. (1993). A population memetics approach to cultural evolution in Chaffinch song: meme diversity within populations. *American Naturalist* **141**, 597–620. doi:10.1086/285493

MacArthur, R. H., and Wilson, E. O. (1967). 'The Theory of Island Biogeography.' (Princeton University Press: Princeton, NJ.)

Marler, P., and Slabbekoorn, H. (2004). 'Nature's Music: The Science of Birdsong.' (Elsevier Academic Press: London.)

Marshall-Ball, L., and Slater, P. J. B. (2004). Duet singing and repertopire use in threat signalling of individuals and pairs. *Proceedings of the Royal*

Society of London – B. Biological Sciences **271**, 440–443. doi:10.1098/rsbl.2004.0186

Mennill, D. J., and Vehrencamp, S. L. (2008). Context-dependent functions of avian duets revealed by microphone-array recordings and multispeaker playback. *Current Biology* **18**, 1314–1319. doi:10.1016/j.cub.2008.07.073

Moravec, M. L., Striedter, G. F., and Burley, N. T. (2006). Assortative pairing based on contact call similarity in Budgerigars, *Melopsittacus undulatus*. *Ethology* **112**, 1108–1116. doi:10.1111/j.1439-0310.2006.01267.x

Morton, E. S. (1975). Ecological sources of selection on avian sounds. *American Naturalist* **109**, 17–34. doi:10.1086/282971

Mulder, R. A. (1995). Natal breeding dispersal in a co-operative, extra-group mating bird. *Journal of Avian Biology* **26**, 234–240. doi:10.2307/3677324

Mulder, R. A., Dunn, P. O., Cockburn, A., Lazenby-Cohen, A., and Howell, M. J. (1994). Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proceedings of the Royal Society of London – B. Biological Sciences* **255**, 223–229. doi:10.1098/rspb.1994.0032

Myers, S., Brown, G. P., and Kleindorfer, S. (2010). Divergence in New Holland Honeyeaters (*Phylidonyris novaehollandiae*): evidence from morphology and feeding behaviour. *Journal of Ornithology* **151**, 287–296. doi:10.1007/s10336-009-0454-7

Myers, S. A., Donnellan, S., and Kleindorfer, S. (2012). Rainfall can explain adaptive phenotypic variation with high gene flow in the New Holland Honeyeater (*Phylidonyris novaehollandiae*). *Ecology and Evolution* **2**, 2397–2412. doi:10.1002/ece3.352

Nowicki, S., and Searcy, W. A. (2005). Song and mate choice in birds: how the development of behavior helps us understand function. *Auk* **122**, 1–14. doi:10.1642/0004-8038(2005)122[0001:SAMCIB]2.0.CO;2

Paton, D. C., Gates, J. A., and Pedler, L. P. (2002). Birds. In 'Natural History of Kangaroo Island'. (Eds M. Davies, C. R. Twidale and M. J. Tyler.) pp. 88–110. (Royal Society of South Australia Inc: Adelaide.)

Peters, A., Kingma, S. A., and Delhey, K. (2013). Seasonal male plumage as a multicomponent sexual signal: insights and opportunities. *Emu* **113**(3), 232–247. doi:10.1071/MU12083

Petrinovich, L., and Patterson, T. L. (1981). The responses of White-crowned Sparrows to songs of different dialects and subspecies. *Zeitschrift für Tierpsychologie* **57**, 1–14. doi:10.1111/j.1439-0310.1981.tb01309.x

Podos, J. (2001). Correlated evolution of morphology and vocal signal structure in Darwin's Finches. *Nature* **409**, 185–188. doi:10.1038/35051570

Podos, J. (2007). Discrimination of geographical song variants by Darwin's Finches. *Animal Behaviour* **73**, 833–844. doi:10.1016/j.anbehav.2006.11.001

Podos, J. (2010). Acoustic discrimination of sympatric morphs in Darwin's Finches: a behavioral mechanism for assortative mating? *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **365**, 1031–1039. doi:10.1098/rstb.2009.0289

Podos, J., and Nowicki, S. (2004). Beaks, adaptation, and vocal evolution in Darwin's Finches. *Bioscience* **54**, 501–510. doi:10.1641/0006-3568(2004)054[0501:BAAVEI]2.0.CO;2

Price, T. (2008). 'Speciation in Birds.' (Roberts and Company Publishers: Greenwood Village, CO.)

Pruett-Jones, S. G., and Lewis, M. J. (1990). Sex ratio and habitat limitation promote delayed dispersal in Superb Fairy-wrens. *Nature* **348**, 541–542. doi:10.1038/348541a0

Ratcliffe, L. M., and Otter, K. (1996). Sex differences in song recognition. In: 'Ecology and Evolution of Acoustic Communication in Birds'. (Eds D. E. Kroodsma and E. H. Miller.) pp 339–355. (Cornell University Press: New York.)

Rowley, I. (1964). The life history of the Superb Blue Wren *Malurus cyaneus*. *Emu* **64**, 251–297. doi:10.1071/MU964251

Rowley, I. C. R., and Russell, E. (1997). 'Fairy-wrens and Grasswrens: Maluridae.' (Oxford University Press: Oxford, UK.)

Schlotfeldt, B. E., and Kleindorfer, S. (2006). Adaptive divergence in the Superb Fairy-wren (*Malurus cyaneus*): a mainland versus island comparison of morphology and foraging behaviour. *Emu* **106**, 309–319. doi:10.1071/MU06004

Schodde, R. (1982). 'The Fairy-wrens. A Monograph of the Maluridae.' (Lansdowne Editions: Melbourne.)

Scholtfeldt, B. E. (2010). Genetic and morphological distinctiveness across Superb Fairy-wren populations in South Australia. Ph. D. Thesis, Flinders University, Adelaide.

Searcy, W. A., and Brenowitz, E. A. (1988). Sexual differences in species recognition of avian song. *Nature* **332**, 152–154. doi:10.1038/332152a0

Searcy, W. A., and Marler, P. (1981). A test for responsiveness to song structure and programming in female sparrows. *Science* **213**, 926–928. doi:10.1126/science.213.4510.926

Searcy, W. A., and Yasukawa, K. (1996). Song and female choice. In 'Animal Signals: Signalling and Signal Design in Animal Communication'. (Eds Y. O. Espmark, T. Amundsen and G. Rosenqvist.) pp. 301–315. (Tapir: Trondheim, Norway.)

Skroblin, A., and Murphy, S. A. (2013). The conservation status of Australian malurids and their value as models to understand management issues. *Emu* **113**(3), 309–318. doi:10.1071/MU12075

Slabbekoorn, H., and Smith, T. B. (2002). Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **357**, 493–503. doi:10.1098/rstb.2001.1056

Slater, P. J. B., and Mann, N. I. (2004). Why do the females of many bird species sing in the tropics? *Journal of Avian Biology* **35**, 289–294. doi:10.1111/j.0908-8857.2004.03392.x

Spencer, K. A., Wimpenny, J. H., Buchanan, K. L., Lovell, P. G., Goldsmith, A. R., and Catchpole, C. K. (2005). Developmental stress affects the attractiveness of male song and female choice in the Zebra Finch (*Taeniopygia guttata*). *Behavioral Ecology and Sociobiology* **58**, 423–428. doi:10.1007/s00265-005-0927-5

Sunnucks, P. (2011). Towards modelling persistence of woodland birds: the role of genetics. *Emu* **111**, 19–39. doi:10.1071/MU10008

Szabo, J. K., Vesk, P. A., Baxter, W. J., and Possingham, H. P. (2011). Paying the extinction debt: woodland birds in the Mount Lofty Ranges, South Australia. *Emu* **111**, 59–70. doi:10.1071/MU09114

Thorpe, W. H., Hall-Craggs, J., Hooker, T., and Hutchinson, R. (1972). Duetting and antiphonal songs in birds: its extent and significance. *Behaviour* **18**, 1–197.

Tobias, J. A., Montgomerie, R., and Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **367**, 2274–2293. doi:10.1098/rstb.2011.0280

van Doorn, G. S., Edelaar, P., and Weissing, F. J. (2009). On the origin of species by natural and sexual selection. *Science* **326**, 1704–1707. doi:10.1126/science.1181661

Webster, M. S., Tarvin, K. A., Tuttle, E. M., and Pruett-Jones, S. (2007). Promiscuity drives sexual selection in a socially monogamous bird. *Evolution* **61**, 2205–2211. doi:10.1111/j.1558-5646.2007.00208.x

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Appendix 2

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Research

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Animal behaviour

Females that experience threat are better teachers

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Superb fairy-wren (*Malurus cyaneus*) females use an incubation call to teach their embryos a vocal password to solicit parental feeding care after hatching. We previously showed that high call rate by the female was correlated with high call similarity in fairy-wren chicks, but not in cuckoo chicks, and that parent birds more often fed chicks with high call similarity. Hosts should be selected to increase their defence behaviour when the risk of brood parasitism is highest, such as when cuckoos are present in the area. Therefore, we experimentally test whether hosts increase call rate to embryos in the presence of a singing Horsfield's bronze-cuckoo (*Chalcites basalis*). Female fairy-wrens increased incubation call rate when we experimentally broadcast cuckoo song near the nest. Embryos had higher call similarity when females had higher incubation call rate. We interpret the findings of increased call rate as increased teaching effort in response to a signal of threat.

1. Introduction

Animals that learn, and hence generate new motor patterns in response to currently perceived information, can rapidly embark on altered evolutionary trajectories [1]. Brood parasites and their hosts are model systems to test different evolutionary trajectories that arise from learning because of strong selection on hosts to avoid cuckoo recognition errors in their nest, and selection on cuckoos to locate suitable host nests. For example, reproductive isolation between indigobird (*Vidua* spp.) brood parasites is maintained because young males learn to mimic their host species' song and young females learn to prefer attributes of the host species nest and the learned song of the conspecific male [2]. Naive juvenile superb fairy-wrens (*Malurus cyaneus*) use social learning to recognize a brood parasite after observing experienced birds mob the cuckoo mount [3]. Recently, Colombelli-Négrel *et al*. [4] showed that learning begins inside the egg: female superb fairy-wrens teach their embryos a vocal password that chicks produce as a begging call after hatching to elicit parental food provisioning [4]. Chicks that had learned the call well, as evidenced by high call similarity after hatching, received more parental provisioning [4]. On the contrary, Horsfield's bronze-cuckoo (*Chalcites basalis*) chicks had low call similarity after hatching, perhaps because the embryos are exposed to the female incubation calls for fewer days or because they use call matching after hatching to elicit food from their foster parents [4,5].

In Australia, high annual fluctuation in brood parasite prevalence in host nests (0–43% across years and study sites [6]) creates selection for risk assessment and dynamic response to changing threat levels [7]. When hosts express defences towards brood parasites, the costs can be high because of recognition errors; hosts may incorrectly identify the eggs and chicks of cuckoos versus their offspring [8–11]. Therefore, hosts should be selected to modify the expression of defence in relation to the risk of parasitism [7,12]. For example, *Acrocephalus* hosts increase mobbing behaviour at the nest when the risk of parasitism is high [13]. But when brood parasite risk is low, hosts accept more foreign eggs into their nest, thereby lowering the rejection threshold for eggs and chicks [12,14]. Here, we test whether incubating female fairy-wrens increase their incubation call rate when they hear the broadcast of a cuckoo near their nest. Cuckoo chicks do not learn as embryos (no evidence to date), whereas fairy-wren embryos do; the presence of a well-learned begging call element is a signal to feed or abandon the brood in this system [4]. This study tests for an early frontline defence mechanism (in this case, teaching embryos) that would thwart the successful fledging of a cuckoo chick [7,8,15].

2. Material and methods

Superb fairy-wrens are common hosts of Horsfield's bronze-cuckoo [6,16]. In South Australia, annual parasite intensity varied from 0 to 37% in 233 nests studied since 2006 (D. Colombelli-Négrel 2014, unpublished data). This study was done during 2007–2013 at Cleland Wildlife Sanctuary (34°58′ S, 138°41′ E) and Newland Head Conservation Park (35°37′ S, 138°29′ E).

We recorded in-nest vocalizations from 17 nests as follows: 2 h of incubation call recordings day 10–11 of incubation and 2 h of chick begging call vocalizations day 4–6 post-hatching. During 2007–2011, the nest recordings were done as described in [4]. In 2012–2013, the recordings were made using a Zoom handy recorder H4n (Zoom Corporation, Australia). We recorded all sound files as broadcast wave files (44.1 kHz sampling rate, 16-bit depth). We edited the recordings with Amadeus Pro v. 1.5 (Hairersoft Inc., Switzerland) and analysed them with Raven Pro v. 1.3 (for methods, see [4]). We noted the number of incubation calls per hour for each female on the basis of acoustical and visual records (spectrograms). An incubation call contains a unique element per female termed the signature element, which is the same element as the chick begging call element after hatching. We calculated call similarity scores per nest for five signature elements per female and five chick begging calls per nest using spectrographic cross-correlation in Raven Pro v. 1.3 and principal coordinates analysis (PCoA); we used R-package software to create PCoA coordinate values (similarity values) [4] and regression analysis to test for number of calls per hour and mean similarity values ('call similarity').

To test whether females adjust incubation call rate to the threat of cuckoo parasitism, we conducted playback trials at 22 nests in 2012 and seven nests in 2013 during the incubation phase (day 10 or 11). We used only one stimulus per nest and broadcast the song of either Horsfield's bronze-cuckoo (at 16 host nests) or striated thornbill (*Acanthiza lineata*; control; at 13 host nests). The playback stimuli (five different Horsfield's bronze-cuckoo, five different striated thornbill) were normalized at −15 dB and saved as uncompressed 16-bit 44.1 kHz broadcast wave files (Amadeus Pro v. 1.5). The playback stimuli consisted of 15 s of song repeated every minute for 1 h. The stimulus track had 1 h of pre-playback silence (pre-trial) followed by 1 h of playback (trial). We broadcast the playback stimuli as uncompressed files from an Apple iPod (Apple Inc., USA) connected to a yo-yo speaker placed 5 m from the nest. We recorded the nest again for 1 h on the following day; all recordings were made using a Zoom handy recorder H4n.

3. Results

Call similarity between female signature element and chick begging call was predicted by the number of incubation calls per hour (linear regression: $r = 0.58$, $t = 2.21$, $p = 0.046$), which we tested in 17 observational nests without exposure to playback stimuli. If females had high incubation call rate, then the call similarity between female signature element (during incubation) and chick begging call element (after hatching) was higher.

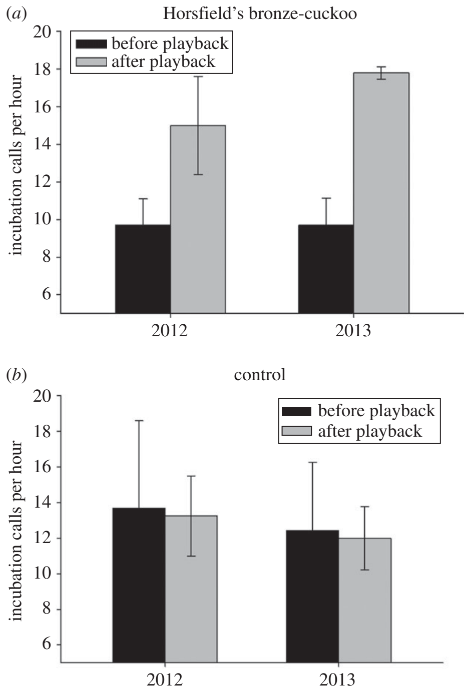

Figure 1. The number of female incubation calls before and after playback of (*a*) cuckoo song ($n = 16$) and (*b*) control ($n = 13$) near the nest across two years of study (2012, 2013). Females increased incubation calls in response to broadcast of the cuckoo song (Horsfield's bronze-cuckoo) but not the control (striated thornbill).

We next compared female incubation call rate at 29 experimental nests before and after exposure to the broadcast of cuckoo and control calls. Before the experiment, there was no significant difference in incubation call rate between nests (park: $F_{1,28} = 0.554$; $p = 0.464$; year: $F_{1,28} = 0.001$; $p = 0.978$; interaction term: $F_{1,28} = 0.084$; $p = 0.775$). There was an effect of the experiment on female call rate: females increased incubation call rate after the broadcast of cuckoo calls (paired *t*-test: $t = -2.936$; d.f. = 15; $p = 0.010$) but not after the broadcast of control calls (striated thornbill; $t = 0.788$; d.f. = 12; $p = 0.446$; figure 1).

4. Discussion

Female superb fairy-wrens increased the number of incubation calls under conditions of perceived threat of brood parasitism. The similarity between begging call and female signature element (call similarity), which is a proxy for embryonic learning outcome and which predicts parental food provisioning of chicks, was higher when females produced more incubation calls [4,17].

Cuckoos call to attract a mate [18]. Upon hearing a cuckoo call, a host should increase vigilance and other anti-parasite behaviours to thwart the cuckoo from successfully fledging [7,15]. Here, we showed that female fairy-wrens that heard a cuckoo near their nest increased call rate to embryos,

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which increased call similarity after hatching, and hence—as we previously showed—increased detectability of an intruder cuckoo chick after hatching. Assuming that cuckoo embryos could learn (for which there is no evidence to date [5]), increased female incubation call rate (which begins late in the incubation phase [4]) would have a lesser effect on cuckoo embryos that hatch days earlier than host embryos (early cuckoo hatching is favoured to evict host eggs). This study provides a mechanistic explanation for how 'experience' with a brood parasite near the territory can lead to increased cuckoo abandonment [19], which is a pattern found across numerous studies [6].

Most studies of host–parasite coevolution focus on visual arms races [20,21]. Our research has shown that acoustical cues are sufficient for fairy-wren hosts to perceive the threat of brood parasitism [6]. Acoustical cues may be more reliable than visual cues to detect the presence of cuckoos. First, visual detection of cuckoos could fail, because cuckoos are discreet while tracking egg-laying hosts, given that hosts will likely mob a detected cuckoo. Second, cuckoos can be similar in appearance to *Accipiter* hawks, thereby creating potential for a costly recognition error [22]. The use of acoustical cues could therefore reduce the chance of mistaking a hawk for a cuckoo while mobbing a bird close to the nest [23]. The use of visual cues can also be misleading for cuckoo detection in the nest. While host nests with a single chick are more likely to be abandoned by fairy-wren parents, it is not always the case that nests with a single chick are cuckoo chicks. Usually, a Horsfield's bronze-cuckoo egg hatches earlier than the host eggs and the newly hatched cuckoo evicts all host eggs in the nest, leaving a single chick [12]. But sometimes a cuckoo egg may hatch after the host chicks have hatched; in these cases, the newly hatched cuckoo chick evicts the somewhat older fairy-wren chicks. Low reproductive success is not uncommon in fairy-wrens (especially during drought years), and may result in a nest with a single fairy-wren chick. While fairy-wren parents are more likely to abandon a single chick, this is an unreliable cue for cuckoo presence in the nest; using a simple visual cue such as 'single chick' increases the risk of mistakenly abandoning one's own sole surviving offspring [6]. Notably, superb fairy-wren parents are more likely to reject a single chick (cuckoo, fairy-wren) when there are cuckoos in the study area [12]—which is evidence that fairy-wrens alter their rejection rules. Here, we show that female fairy-wrens increase incubation call rate when there are cuckoos in the area, which we argue would lower the probability of committing an acceptance error for a cuckoo, or a rejection error for a fairy-wren chick.

The so-called frontline of defence in host and brood parasite systems is well supported by evidence [15], including cuckoo mobbing [8], egg mimicry [24] and begging call learning [25]. This study shows that female fairy-wrens increased teaching effort to embryos after receiving acoustical information about the threat of brood parasitism during incubation. In a separate study, we showed increased predation risk at nests with high incubation call rate [26], which likely explains why female fairy-wrens only increase call rate when the risk of cuckoo parasitism is high. The benefits to the embryos of learning could be numerous, including a lifelong trajectory of learning in unpredictable environments [27]. These findings add to a growing body of empirical evidence that animals teach naive individuals, such as offspring [28–32], and that hosts modify the expression of defence in relation to risk, including risk of parasitism [11]. What is novel about this study is that we show changes in host teaching effort (call rate) and host learning outcome (call similarity) under conditions of brood parasite threat.

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References

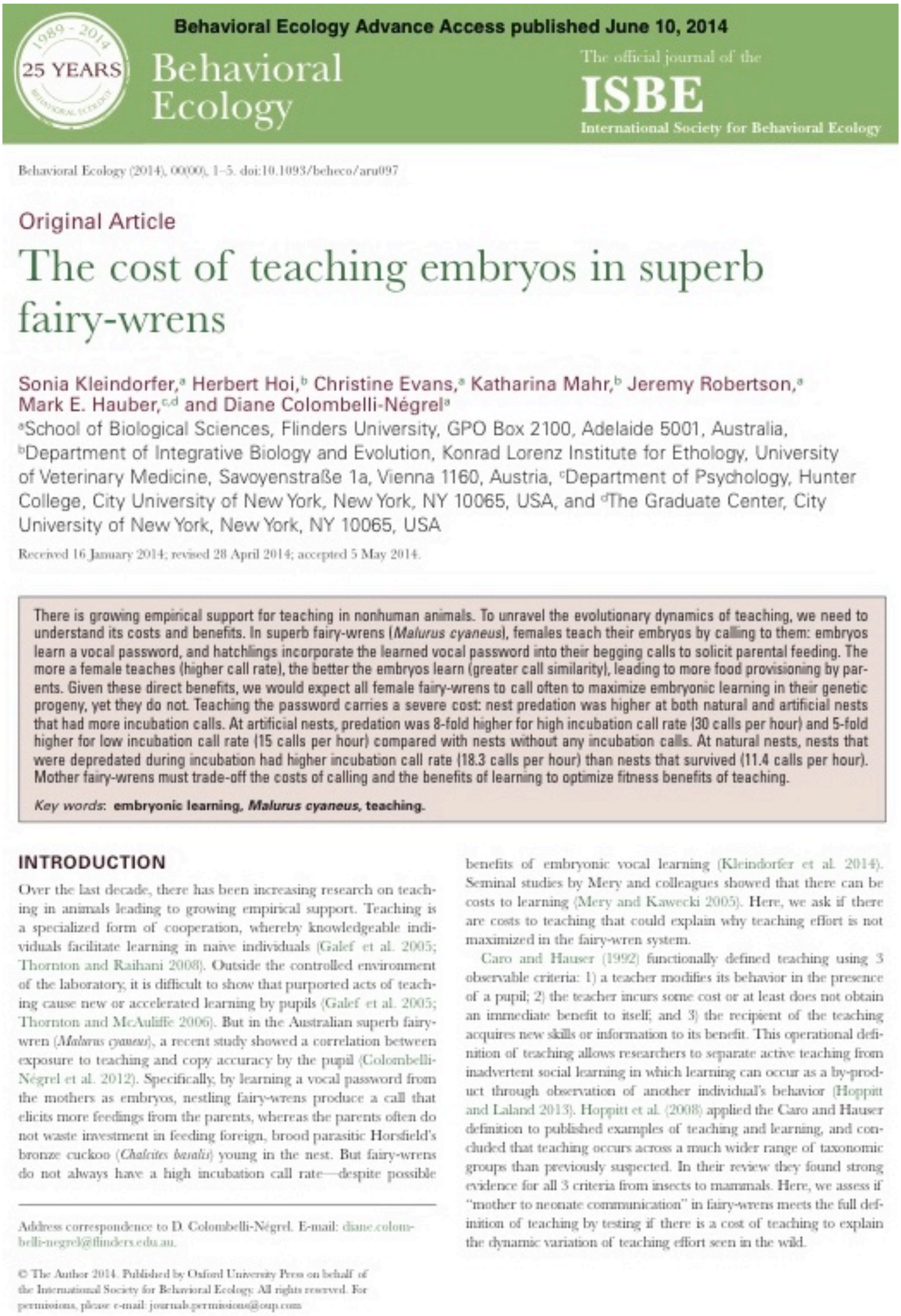
1. Dukas R. 2013 Effects of learning on evolution: robustness, innovation and speciation. *Anim. Behav.* **85**, 1023–1030. (doi:10.1016/j.anbehav.2012.12.030)
2. Sorenson MD, Sefc KM, Payne RB. 2003 Speciation by host switch in brood parasitic indigobirds. *Nature* **424**, 928–931. (doi:10.1038/nature01863)
3. Feeney WE, Langmore NE. 2013 Social learning of a brood parasite by its host. *Biol. Lett.* **9**, 20130443. (doi:10.1098/rsbl.2013.0443)
4. Colombelli-Négrel D, Hauber ME, Robertson J, Sulloway FJ, Hoi H, Griggio M, Kleindorfer S. 2012 Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Curr. Biol.* **22**, 2155–2160. (doi:10.1016/j.cub.2012.09.025)
5. Langmore NE, Maurer G, Adcock GJ, Kilner RM. 2008 Socially acquired host-specific mimicry and the evolution of host races in Horsfield's bronze-cuckoo *Chalcites basalis*. *Evolution* **62**, 1689–1699. (doi:10.1111/j.1558-5646.2008.00405.x)
6. Langmore N. 2013 Fairy-wrens as a model system for studying cuckoo host coevolution. *Emu* **113**, 302–308. (doi:10.1071/MU12079)
7. Kleindorfer S, Evans C, Colombelli-Négrel D, Robertson J, Griggio M, Hoi H. 2013 Host response to cuckoo song is predicted by the future risk of brood parasitism. *Front. Zool.* **10**, 30. (doi:10.1186/1742-9994-10-30)
8. Trnka A, Prokop P, Grim T. 2012 Uncovering dangerous cheats: how do avian hosts recognize adult brood parasites? *PLoS ONE* **7**, e37445. (doi:10.1371/journal.pone.0037445)
9. Grim T, Samaš P, Moskát C, Kleven O, Honza M, Moksnes A, Røskaft E, Stokke BG. 2011 Constraints on host choice: why do parasitic birds rarely exploit some common potential hosts? *J. Anim. Ecol.* **80**, 508–518. (doi:10.1111/j.1365-2656.2010.01798.x)
10. Grim T. 2006 The evolution of nestling discrimination by hosts of parasitic birds: why is rejection so rare? *Evol. Ecol. Res.* **8**, 785–802.
11. Davies NB. 2011 Cuckoo adaptations: trickery and tuning. *J. Zool.* **284**, 1–14. (doi:10.1111/j.1469-7998.2011.00810.x)
12. Langmore NE, Cockburn A, Russell AF, Kilner RM. 2009 Flexible cuckoo chick-rejection rules in the superb fairy-wren. *Behav. Ecol.* **20**, 978–984. (doi:10.1093/beheco/arp086)
13. Welbergen JA, Davies NB. 2009 Strategic variation in mobbing as a front line of defense against brood parasitism. *Curr. Biol.* **19**, 235–240. (doi:10.1016/j.cub.2008.12.041)
14. Grim T. 2007 Experimental evidence for chick discrimination without recognition in a brood parasite host. *Proc. R. Soc. B* **274**, 373–381. (doi:10.1098/rspb.2006.3731)
15. Feeney WE, Welbergen JA, Langmore NE. 2012 The frontline of avian brood parasite–host coevolution. *Anim. Behav.* **84**, 3–12. (doi:10.1016/j.anbehav.2012.04.011)
16. Brooker MG, Brooker L. 1989 Cuckoo hosts in Australia. *Aust. Zool. Rev.* **2**, 1–67.

2112

2113

17. Langmore NE, Hunt S, Kilner RM. 2003 Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature* **422**, 157–160. (doi:10.1038/nature01460)
18. Fuisz TI, De Kort SR. 2007 Habitat-dependent call divergence in the common cuckoo: is it a potential signal for assortative mating? *Proc. R. Soc. B* **274**, 2093–2097. (doi:10.1098/rspb.2007.0487)
19. Langmore N, Feeney W, Crowe-Riddell J, Luan H, Louwrens K, Cockburn A. 2012 Learned recognition of brood parasitic cuckoos in the superb fairy-wren *Malurus cyaneus*. *Behav. Ecol.* **23**, 798–805. (doi:10.1093/beheco/ars033)
20. Davies NB, Brooke MD. 1989 An experimental-study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. 1. Host egg discrimination. *J. Anim. Ecol.* **58**, 207–224. (doi:10.2307/4995)
21. Langmore NE, Stevens M, Maurer G, Heinsohn R, Hall ML, Peters A, Kilner RM. 2011 Visual mimicry of host nestlings by cuckoos. *Proc. R. Soc. B* **278**, 2455–2463. (doi:10.1098/rspb.2010.2391)
22. Welbergen JA, Davies NB. 2011 A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts. *Behav. Ecol.* **22**, 574–579. (doi:10.1093/beheco/arr008)
23. Trnka A, Prokop P. 2011 The effectiveness of hawk mimicry in protecting cuckoos from aggressive hosts. *Anim. Behav.* **83**, 263–268. (doi:10.1016/j.anbehav.2011.10.036)
24. Stoddard MC, Stevens M. 2010 Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. B* **277**, 1387–1393. (doi:10.1098/rspb.2009.2018)
25. Roldán M, Soler M, Márquez R, Soler JJ. 2013 The vocal begging display of great spotted cuckoo *Clamator glandarius* nestlings in nests of its two main host species: genetic differences or developmental plasticity? *Ibis* **155**, 867–876. (doi:10.1111/ibi.12088)
26. Kleindorfer S, Hoi H, Evans C, Mahr K, Robertson J, Hauber ME, Colombelli-Négrel D. In press. The cost of teaching embryos in superb fairy-wrens. *Behav. Ecol.*
27. Kasumovic MM, Brooks RC. 2011 It's all who you know: the evolution of socially cued anticipatory plasticity as a mating strategy. *Q. Rev. Biol.* **86**, 181–197. (doi:10.1086/661119)
28. Hoppitt WJ, Brown GR, Kendal R, Rendell L, Thornton A, Webster MM, Laland KN. 2008 Lessons from animal teaching. *Trends Ecol. Evol.* **23**, 486–493. (doi:10.1016/j.tree.2008.05.008)
29. Thornton A, McAuliffe K. 2012 Teaching can teach us a lot. *Anim. Behav.* **83**, e6–e9. (doi:10.1016/j.anbehav.2012.01.029)
30. Byrne RW, Rapaport LG. 2011 What are we learning from teaching? *Anim. Behav.* **82**, 1207–1211. (doi:10.1016/j.anbehav.2011.08.018)
31. Strauss S, Ziv M. 2011 What nonhuman animal teaching teaches us about teaching. In *CARLS series of advanced studies of logic and sensibility*, vol. 5 (ed. J. Ando), pp. 363–371. Tokyo, Japan: Keio University Press.
32. Raihani NJ, Ridley AR. 2008 Experimental evidence for teaching in wild pied babblers. *Anim. Behav.* **75**, 3–11. (doi:10.1016/j.anbehav.2007.07.024)

Appendix 3



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Original Article

The cost of teaching embryos in superb fairy-wrens

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There is growing empirical support for teaching in nonhuman animals. To unravel the evolutionary dynamics of teaching, we need to understand its costs and benefits. In superb fairy-wrens (*Malurus cyaneus*), females teach their embryos by calling to them: embryos learn a vocal password, and hatchlings incorporate the learned vocal password into their begging calls to solicit parental feeding. The more a female teaches (higher call rate), the better the embryos learn (greater call similarity), leading to more food provisioning by parents. Given these direct benefits, we would expect all female fairy-wrens to call often to maximize embryonic learning in their genetic progeny, yet they do not. Teaching the password carries a severe cost: nest predation was higher at both natural and artificial nests that had more incubation calls. At artificial nests, predation was 8-fold higher for high incubation call rate (30 calls per hour) and 5-fold higher for low incubation call rate (15 calls per hour) compared with nests without any incubation calls. At natural nests, nests that were depredated during incubation had higher incubation call rate (18.3 calls per hour) than nests that survived (11.4 calls per hour). Mother fairy-wrens must trade-off the costs of calling and the benefits of learning to optimize fitness benefits of teaching.

Key words: **embryonic learning, *Malurus cyaneus*, teaching.**

INTRODUCTION

Over the last decade, there has been increasing research on teaching in animals leading to growing empirical support. Teaching is a specialized form of cooperation, whereby knowledgeable individuals facilitate learning in naive individuals (Galef et al. 2005; Thornton and Raihani 2008). Outside the controlled environment of the laboratory, it is difficult to show that purported acts of teaching cause new or accelerated learning by pupils (Galef et al. 2005; Thornton and McAuliffe 2006). But in the Australian superb fairy-wren (*Malurus cyaneus*), a recent study showed a correlation between exposure to teaching and copy accuracy by the pupil (Colombelli-Négrel et al. 2012). Specifically, by learning a vocal password from the mothers as embryos, nestling fairy-wrens produce a call that elicits more feedings from the parents, whereas the parents often do not waste investment in feeding foreign, brood parasitic Horsfield's bronze cuckoo (*Chalcites basalis*) young in the nest. But fairy-wrens do not always have a high incubation call rate—despite possible benefits of embryonic vocal learning (Kleindorfer et al. 2014). Seminal studies by Mery and colleagues showed that there can be costs to learning (Mery and Kawecki 2005). Here, we ask if there are costs to teaching that could explain why teaching effort is not maximized in the fairy-wren system.

Caro and Hauser (1992) functionally defined teaching using 3 observable criteria: 1) a teacher modifies its behavior in the presence of a pupil; 2) the teacher incurs some cost or at least does not obtain an immediate benefit to itself; and 3) the recipient of the teaching acquires new skills or information to its benefit. This operational definition of teaching allows researchers to separate active teaching from inadvertent social learning in which learning can occur as a by-product through observation of another individual's behavior (Hoppitt and Laland 2013). Hoppitt et al. (2008) applied the Caro and Hauser definition to published examples of teaching and learning, and concluded that teaching occurs across a much wider range of taxonomic groups than previously suspected. In their review they found strong evidence for all 3 criteria from insects to mammals. Here, we assess if "mother to neonate communication" in fairy-wrens meets the full definition of teaching by testing if there is a cost of teaching to explain the dynamic variation of teaching effort seen in the wild.

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In line with this, brood parasite–host systems offer another possibility to examine aspects of animal teaching and learning. For example, in birds, brood parasites lay their eggs in the nests of hosts. From the hosts' perspective, there are a range of defense mechanisms to thwart brood parasites, including the rejection or abandonment of foreign eggs and chicks, or front line defenses such as socially learned mobbing of cuckoos to altogether prevent parasitic egg laying (Feeney and Langmore 2013; Feeney et al. 2013). But each of these defense tactics carries the costly risk of recognition errors (Grim 2007; Trnka et al. 2012; Langmore 2013) which could be reduced through teaching and learning. Superb fairy-wrens and their brood parasite are a particularly good model system to investigate this aspect because 1) female superb fairy-wrens actively teach their embryos a vocal password within an incubation call that the hatchlings must emulate in their begging call in order to be fed at high rates, as previous experimental study showed lower food provisioning by parents at nests with low call similarity in chicks (Colombelli-Négrel et al. 2012). The more often the female calls to the embryo the higher the call similarity after hatching (Colombelli-Négrel et al. 2012; Kleindorfer et al. 2014). 2) In the superb fairy-wren we experimentally showed that the begging calls of cross-fostered fairy-wrens are learned rather than innate, because the begging call element more closely resembled the vocal password (element of the incubation call) of their foster mother rather than their genetic mother (Colombelli-Négrel et al. 2012). 3) Embryos of the fairy-wrens' primary brood parasite, Horsfield's bronze cuckoo, have a shorter incubation duration, are hence exposed to the fairy-wren incubation calls for less time, and they also have lower call similarity after hatching (Colombelli-Négrel et al. 2012). And 4) adult fairy-wrens respond to adult cuckoo calls according to the risk of brood parasitism (Kleindorfer et al. 2013). The finding of increased alarm calls and defense behavior by fairy-wrens in response to acoustical cues of different brood parasites in their territory indicates that host fairy-wrens modify their behavior toward cuckoo species, including their investment in teaching effort of embryos (Kleindorfer et al. 2014).

The superb fairy-wren and Horsfield's bronze cuckoo system satisfies 2 of 3 teaching criteria (Caro and Hauser 1992). First, female fairy-wrens increase their teaching effort (call rate) when the naive pupils (the embryos) are more developed, during late-stage incubation (criterion 1; Colombelli-Négrel et al. 2012). Second, learning has fitness benefits because parents feed hatchlings more when they have more accurately learned the vocal password and incorporated it into their begging call compared with hatchlings with lower call similarity, which may include cuckoos (criterion 3). But if teaching is only beneficial, then why is it not more prevalent? The fact that fairy-wren mothers modify teaching effort according to the risk of brood parasitism (e.g., cuckoo presence) (Kleindorfer et al. 2014) suggests that mother to embryo communication may bear costs. One potential cost of acoustic education of embryos in the nest could be that the sound of the incubation call can attract predators and lead to increased nest predation. There is ample evidence from studies of nestling begging calls that vocalization behavior increases predation risk (Haskell 1994; Briskie et al. 1999) and that parental calling (but not visual displays) attracts neighbors and predators (Grim 2008). Consequently, female investment into incubation call production may be the result of a trade-off between benefits from teaching and increased predation risk.

Here, we use observational and experimental approaches to test whether superb fairy-wrens incur a fitness cost from teaching embryos (criterion 2). At natural nests, we recorded incubation calls by female fairy-wrens and compared incubation call rate with nest predation across study years. At artificial nests, we experimentally broadcast incubation calls at different rates and compared predation at nests that differed in number of incubation calls per hour. Finally, we discuss possible evolutionary outcomes when teaching mothers are faced with conditions of high predation and brood parasitism risk.

MATERIALS AND METHODS

Study sites and species

We monitored superb fairy-wren nests and recorded female incubation calls and nest predation during the fairy-wrens' peak breeding period from September to January in the years 2007, 2009–2012. We conducted experimental trials in 2013 to test if artificial nests with incubation calls had a higher proportion of depredated nests outcome than artificial nests without incubation calls. The 2 study sites were in South Australia at: 1) Cleland Wildlife Sanctuary (34°58′S, 138°41′E), 25 kms SE of Adelaide; and 2) Newland Head Conservation Park (35°37′S, 138°29′E), a coastal area 15 kms SW of Victor Harbor on the Fleurieu Peninsula.

Nest predation

Between 2007 and 2009–2012, we located 138 nests and monitored them every 3 days for nesting outcome (fledging, predation, abandonment) (Table 1). For this study, we only test incubation call rate in relation to nesting outcome for nests that were observed during the incubation phase (n = 42 nests with incubation call rate and predation outcome). Predation was scored if eggs were missing from the nest between the nest content examinations every 3 days.

Recording incubation call rate across years

Between 2007 and 2009–2012, we recorded 428 female incubation calls from 42 nests. The methods to record the in-nest vocalizations between 2007, 2009–2011 are given in Colombelli-Négrel et al. (2012). In 2012, we recorded all vocalizations at the nest for 2 h on days 10–11 of incubation using a Zoom Handy Recorder H4n (Zoom Corporation, Tokyo, Japan); sound files were recorded as broadcast wave files at 48 kHz sampling rate, 24-bit depth. We transcribed each recorded vocalization to an Apple MacPro (Apple Corporation, Sydney, Australia) for editing with Amadeus Pro 1.5 (Hairersoft Inc., Kenilworth, UK). For this study, we analyzed the number of incubation calls per hour (call rate) in relation to

Table 1

Incubation call rate and predation outcome differ across years

Study site and year	Number of incubation calls per hour (n = 42)	Nest predation (n = 138)
Newland Head		
2007	13.9 ± 4.8	57% (8/14)
2009	23.3 ± 5.6	83% (5/6)
2011	10.9 ± 1.1	56% (18/32)
2012	9.8 ± 3.6	53% (10/19)
Cleland		
2011	17.6 ± 0.2	40% (8/20)
2012	10 ± 1.4	34% (16/47)

Annual differences in number of incubation calls per hour (mean ± standard error) and nest predation (%) are shown for Newland Head Conservation Park and Cleland Conservation Park in South Australia.

predation outcome. We compared call rate per female based on 2h of recording for 2 consecutive days per female.

Broadcast of incubation calls at artificial nests

In October and November 2013, we broadcast incubation calls at artificial nests to test whether incubation calls elevate predation risk. We used domed woven nests baited with a single domestic Japanese quail (*Coturnix japonica*) and for 5h (0600 to 1100) at every nest including control nests, we placed a battery powered amplified speaker connected to an Apple iPod (Apple Inc., Sydney, Australia). The experiment was repeated every day for 3 days. Three days is the average number of days a female calls to a late-stage embryos in the nest (females call to cuckoo eggs for ~2 days and to wren eggs for ~4 days). After the 5h we checked every nest for predation and collected the playback equipment. The nests were placed 30–50cm above the ground every 100 m along 5 transects at Newland Head Conservation Park. At every second nest, we broadcast a female incubation call. Each transect had 20 nests for a total of 100 nests: 30 nests with a high call rate (30 incubation calls per hour), 20 nests with a low call rate (15 incubation calls per hour), and 50 control nests with no incubation call. The natural range in mean incubation call rate across years in South Australia was 10–23 incubation calls per hour (Table 1). The playback stimuli were the incubation calls of 4 fairy-wrens that had been recorded in 2009. We normalized the playbacks at −6 dB and saved them as uncompressed 24-bit 48kHz broadcast wave files using Amadeus Pro 1.5.

To test if nest concealment due to vegetation cover differed between experimental (47), control (50), and wild (22) nests, we compared the proportion of the nest that was concealed by vegetation between the 3 treatment groups. None of the nest-site vegetation scores differed significantly across the 3 groups: vegetation cover 1 m in front of the nest (Anova: $F = 1.322$, df = 2, $P = 0.270$), vegetation cover 1 m to the left of the nest ($F = 2.110$, df = 2, $P = 0.110$), vegetation cover 1 m to the right of the nest ($F = 1.139$, df = 2, $P = 0.323$), or nest height (cm) measured from the ground to the entrance hole ($F = 2.056$, df = 2, $P = 0.138$). None of the pairwise post-hoc comparisons was significantly different for vegetation cover between experimental, control, and natural nests.

Ethics statement

This study on wild superb fairy-wrens was approved by the Animal Welfare Committee of Flinders University (E234-236) and supported by a scientific permit to conduct the research (Z24699 4).

Statistical analysis

Data were analyzed with SPSS 20 for Windows (SPSS Inc., Chicago, IL). We used multivariate analysis of variance (MANOVA) to analyze incubation call rate and annual predation per year with study site as a random factor. We used binary logistic regression to test if number of incubation calls per hour per nest was correlated with predation outcome (survived, predated) at natural nests, and a chi-squared test for the effect of incubation call rate on predation outcome at artificial nests.

RESULTS

Both nest predation and number of incubation calls per hour differed significantly across study years in South Australia (MANOVA: predation outcome: $F_{3,41} = 231.77$, $P < 0.001$; call rate: $F_{1,41} = 3.82$, $P = 0.018$). The range in percentage of depredated nests was 34–83% across study years, and the range in mean number of incubation calls per hour was 10–23 (Table 1). To test if incubation call rate increased with the risk of predation at natural nests, we examined predation outcome during the incubation phase (survived, depredated) against the number of incubation calls per hour. Nest predation was significantly higher when females produced more incubation calls per hour (binary logistic regression: odds = 0.35, $P = 0.023$, $n = 42$; Figure 1). Mean number of incubation calls per hour at nests that survived was 11.4 ± 1.4 ($n = 33$) compared with 18.3 ± 2.5 at nests that were depredated ($n = 9$) (Figure 1).

Experimental incubation calls and predation outcome

At experimental nests, nest predation was higher when incubation call rate was higher ($\chi^2 = 30.39$, df = 2.100, $P < 0.001$; Figure 2). Of the 30 nests with high incubation call rate, 20 were depredated (67%). Of the 20 nests with low incubation call rate, 8 were depredated (40%). Of the 50 control nests, 4 were depredated (8%). Thus, predation was 8-fold higher at nests with high incubation call rate (8% vs. 67%), and 5-fold higher at nests with low incubation call rate (8% vs. 40%). In most cases, the predation events occurred during the 5-h playback trial (during 18/20 cases for high incubation call rate, 4/8 cases for low incubation call rate, and 0/4 cases for no incubation calls) ($\chi^2 = 7.1$, df = 1.36, $P = 0.008$).

DISCUSSION

We have previously shown that superb fairy-wren embryos learn a vocal password by listening to their mother's incubation call and base their begging calls on the unique vocal password taught to them by the female (Colombelli-Négrel et al. 2012). One function of this teaching and learning is for parents to recognize host hatchlings and expose intruder cuckoo chicks that use call matching after hatching rather than call learning before hatching to emulate the host begging call (Langmore et al. 2008; Colombelli-Négrel et al. 2012). We previously used an experimental approach to show that

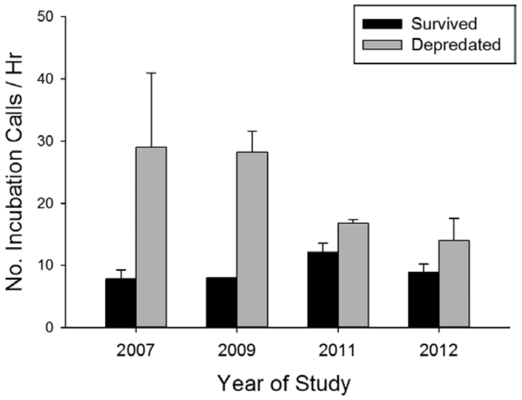

Figure 1
More predation at natural nests with many incubation calls. Nest predation at natural nests is shown in relation to female teaching effort (number of incubation calls) by superb fairy-wren females ($n = 42$ nests). Nest predation was higher at nests with many incubation calls (mean ± standard error). The data are pooled for nests from Newland Head Conservation Park and Cleland Conservation Park, South Australia (Table 1).

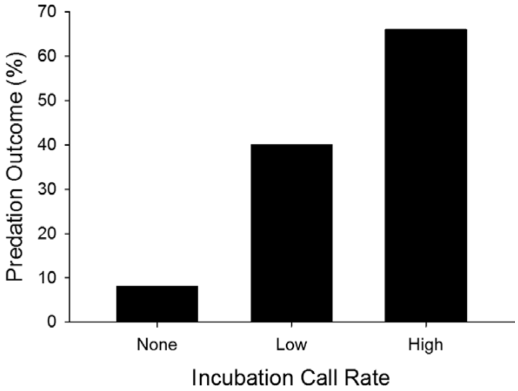

Figure 2
More predation at artificial nests with many incubation calls. Artificial nests were baited with quail eggs and each nest had an iPod and speaker for 5 h. There were 3 treatment groups: 1) no incubation calls (playback equipment present but inactive, *n* = 50); 2) low incubation call rate (15 calls per hour, *n* = 20); or 3) high incubation call rate (30 calls per hour, *n* = 30).

females increased their teaching effort (call rate and vocal password rate) when they heard a cuckoo calling near the nest (Kleindorfer et al. 2014). Here, we show that teaching in this system also carries a potentially high cost as the risk of predation was much higher when there were many incubation calls at both natural and artificial nests. Across years, predation was higher when incubation call rates were higher. At depredated natural nests females produced ~20 incubation calls (18.3) per hour, whereas at nests that survived females produced ~10 incubation calls (11.4) per hour. In experimental nests, predation was 8 times more likely if females had high incubation call rate (30 calls per hour) compared with nests without incubation calls, whereas nests with low incubation call rate (15 calls per hour) were 5 times more likely to be predated. Notably, most predation at artificial nests occurred during the 5-h experimental broadcast (see also Grim 2008). Thus, call rate, and not some other nest-site feature, was the primary cue used by a predator to locate the nest.

Female incubation calling and embryonic learning in the superb fairy-wren system now satisfies all 3 criteria for the operational definition of teaching: 1) modified behavior by the teacher in the presence of a naive observer; 2) a cost to the teacher; and 3) a benefit to the pupil (Caro and Hauser 1992). These findings of teaching and learning in the superb fairy-wren should spur more comparative and field research into the evolution of teaching and learning. For example, females that invest in their offspring via teaching may also have invested other nutrients or resources to promote condition-dependent song learning (Buchanan 2000, 2011; Nowicki et al. 2002a, 2002b; Gil et al. 2006), as has been found in the maternal effects on the learning of song and human language in embryos and neonates (Qvarnström and Price 2001; Spencer et al. 2003; Lipkind et al. 2013). Thus, teaching might be an important but greatly ignored aspect of maternal investment (Fox and Mousseau 1998).

Socially Cued Anticipatory Plasticity is the term given to behavioral plasticity in response to environmental and social cues that alter an individual's morphology, behavior, or life-history strategy (Kasumovic and Brooks 2011). Teaching is one social mechanism that facilitates learning and phenotypic change associated with learning (Hoppitt and Laland 2013). There is growing evidence across taxa from insects to birds and primates that animals teach, and evidence that teachers modify their behavior according to prevailing social and environmental conditions (e.g., Kleindorfer et al. 2014). In this manner, teaching is a social learning mechanism to facilitate the transfer of essential skills from teacher to the next generation (Feeney and Langmore 2013; Hoppitt and Laland 2013). Although many studies focus on the benefits of teaching and learning, this study shows the costs of teaching in the superb fairy-wren system.

Given we have shown teaching has high costs we conclude that teachers should be selected to evaluate the costs and benefits of teaching in dynamic environments. The costs of teaching include mortality risk to adults and/or offspring, such as predation of adults and/or chicks, or brood parasite-induced mortality of chicks (Wyllie 1981; Thornton and Raihani 2008; Anderson et al. 2009; Colombelli-Négrel and Kleindorfer 2009; Fogarty et al. 2011). The benefits of teaching may be different for the teacher (in relation to parent–offspring relatedness) and pupil (impact of learning for survival and/or reproductive success). We suggest that across study populations, and other species of cuckoo hosts, fairy-wrens may strategically adjust their teaching effort (call rate and/or vocal password rate). The following predictions can be formulated: 1) low teaching effort (low call rate) in areas with high predation risk; 2) high teaching effort (high call rate and/or vocal password rate) in areas with high brood parasite risk; and 3) evolutionary adaptations toward making teaching more efficient in systems where both predation and parasite risk is high or in areas where cuckoos are also nest predators. A solution to this dilemma could be to increase the password rate (begging call element within the incubation call) while decreasing call rate and/or call complexity.

Thus, the adaptive interplay between teaching and learning generates testable predictions for other systems with mother to egg teaching. There are 28 species of wrens in the Maluridae, of which 4/4 species of fairy-wren studied to date have female incubation calls (Colombelli-Négrel D et al. unpublished data). When extrapair copulations are frequent, as often occurs in the Maluridae (Cockburn et al. 2013), we also predict different evolutionary selection on putative mothers and fathers. In these circumstances, males and females have different certainty of relatedness to their brood and hence should evaluate the costs and benefits of teaching differently.

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REFERENCES

Anderson MG, Ross HA, Brunton DH, Hauber ME. 2009. Begging call matching between a specialist brood parasite and its host: a comparative approach to detect coevolution. Biol J Linn Soc. 98:208–216.
Briskie JV, Martin PR, Martin TE. 1999. Nest predation and the evolution of nestling begging calls. Proc R Soc B Biol Sci. 266:2153–2159.

Buchanan KL. 2000. Stress and the evolution of condition-dependent signals. Trends Ecol Evol. 15:156–160.

Buchanan KL. 2011. The developmental stress hypothesis: a special case of the evolution of condition-dependent sexual traits. Behav Ecol. 22:12–13.

Caro TM, Hauser MD. 1992. Is there teaching in nonhuman animals? Q Rev Biol. 67:151–174.

Cockburn A, Brouwer L, Double MC, Margraf N, van de Pol M. 2013. Evolutionary origins and persistence of infidelity in Malurus: the least faithful birds. Emu. 113:208–217.

Colombelli-Négrel D, Hauber ME, Robertson J, Sulloway FJ, Hoi H, Griggio M, Kleindorfer S. 2012. Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. Curr Biol. 22:2155–2160.

Colombelli-Négrel D, Kleindorfer S. 2009. Nest height, nest concealment, and predator type predict nest predation in superb fairy-wrens (Malurus cyaneus). Ecol Res. 24:921–928.

Feeney WE, Langmore NE. 2013. Social learning of a brood parasite by its host. Biol Lett. 9:20130443.

Feeney WE, Medina I, Somveille M, Heinsohn R, Hall ML, Mulder RA, Stein JA, Kilner RM, Langmore NE. 2013. Brood parasitism and the evolution of cooperative breeding in birds. Science. 342:1506–1508.

Fogarty L, Strimling P, Laland KN. 2011. The evolution of teaching. Evolution. 65:2760–2770.

Fox CW, Mousseau TA. 1998. Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. In: Fox CW, Mousseau TA, editors. Maternal effects as adaptations. p. 159–177.

Galef BG, Whiskin EE, Dewar G. 2005. A new way to study teaching in animals: despite demonstrable benefits, rat dams do not teach their young what to eat. Anim Behav. 70:91–96.

Gil D, Naguib M, Riebel K, Rutstein A, Gahr M. 2006. Early condition, song learning, and the volume of song brain nuclei in the zebra finch (Taeniopygia guttata). J Neurobiol. 66:1602–1612.

Grim T. 2007. Equal rights for chick brood parasites. Ann Zool Fennici. 44:1–7.

Grim T. 2008. Are Blackcaps (Sylvia atricapilla) defending their nests also calling for help from their neighbours? J Ornithol. 149:169–180.

Haskell D. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. Proc R Soc B Biol Sci. 257:161–164.

Hoppitt WJ, Brown GR, Kendal R, Rendell L, Thornton A, Webster MM, Laland KN. 2008. Lessons from animal teaching. Trends Ecol Evol. 23:486–493.

Hoppitt W, Laland KN. 2013. Social learning: an introduction to mechanisms, methods, and models. Princeton University Press.

Kasumovic MM, Brooks RC. 2011. It's all who you know: the evolution of socially cued anticipatory plasticity as a mating strategy. Q Rev Biol. 86:181–197.

Kleindorfer S, Evans C, Colombelli-Négrel D, Robertson J, Griggio M, Hoi H. 2013. Host response to cuckoo song is predicted by the future risk of brood parasitism. Front Zool. 10:30.

Kleindorfer S, Evans C, Colombelli-Négrel D. 2014. Females that experience threat are better teachers. Biol Lett. 10:20140046.

Langmore N. 2013. Fairy-wrens as a model system for studying cuckoo-host coevolution. Emu. 113:302–308.

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

Lipkind D, Marcus GF, Bemis DK, Sasahara K, Jacoby N, Takahasi M, Suzuki K, Feher O, Ravbar P, Okanoya K, et al. 2013. Stepwise acquisition of vocal combinatorial capacity in songbirds and human infants. Nature. 498:104–108.

Mery F, Kawecki TJ. 2005. A cost of long-term memory in Drosophila. Science. 308:1148.

Nowicki S, Searcy WA, Peters S. 2002a. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 188:1003–1014.

Nowicki S, Searcy WA, Peters S. 2002b. Quality of song learning affects female response to male bird song. Proc Biol Sci. 269:1949–1954.

Qvarnström A, Price TD. 2001. Maternal effects, paternal effects and sexual selection. Trends Ecol Evol. 16:95–100.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of developmental stress in the zebra finch (Taeniopygia guttata). Horm Behav. 44:132–139.

Thornton A, Raihani NJ. 2008. The evolution of teaching. Anim Behav. 75:1823–1836.

Thornton A, McAuliffe K. 2006. Teaching in wild meerkats. Science. 313:227–229.

Wyllie I. 1981. The cuckoo. London: B. T. Batsford Ltd; New York (NY): Universe Books. p. 176.

Trnka A, Prokop P, Grim T. 2012. Uncovering dangerous cheats: how do avian hosts recognize adult brood parasites? PLoS One. 7:e37445.

Appendix 4

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ORIGINAL RESEARCH
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Multiple Ornaments—Multiple Signaling Functions? The Importance of Song and UV Plumage Coloration in Female Superb Fairy-wrens (*Malurus cyaneus*)

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Showy ornaments are considered as outcomes of sexual selection processes. They provide a "badge of status" to impress conspecific rivals or potential mating partners. Single ornaments may signal attractiveness or individual quality, yet many species display multiple ornaments. There are several hypotheses that explain the existence of multiple ornaments, suggesting that different ornaments serve as different information sources. They may provide either additive or redundant information on the same quality traits, or are simply evolutionary leftovers with no further relevant information. Although, females of many species display elaborated traits, most studies regarding multiple ornaments focus on males. However, given that in many species females also display multiple ornaments, the question about their functional significance arises. To understand the existence of female multiple ornaments we investigated ornamental features of female Superb Fairy-wrens (*Malurus cyaneus*), focusing on song and variation in plumage characteristics. Female Superb Fairy-wrens produce complex solo songs, for territorial defense, and have bright blue tail feathers. We examined the relationships between song and plumage coloration characteristics in relation to female quality parameters to investigate whether, and to what extent existing hypotheses on multiple ornaments in males may also apply to females. Based on song recordings and spectrometric measurements of UV-coloration of tail feathers, we derived a series of different song and plumage parameters. Our results indicate interrelationships between the song length (total number of elements in female song) and female body size, but not UV-coloration. Interestingly, song complexity (number of different elements in female song) did not correlate with morphological parameters, UV-Chroma and song length, respectively. This suggests that (i) song and plumage characteristics evolved independently and (ii) even within one trait, namely song, multiple signaling should be considered. To our knowledge, this is the first study investigating multiple traits in female songbirds, raising the idea that multiple signaling of sexually selected traits is not restricted to males only.

Keywords: female, passerines, plumage coloration, song elaboration, multiple signals

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INTRODUCTION

Showy plumage characteristics and elaborated song in passerines are known to be typical male traits, shaped by sexual selection (Andersson, 1994). There is strong evidence that both traits signal individual quality and are therefore involved in mate choice, as well as competitive interactions (Burley, 1986; Searcy and Andersson, 1986; Andersson, 1994; Nowicki and Searcy, 2004; Hoi and Griggio, 2008). However, the expression of colorful plumage and elaborated song is not restricted to males; females can also display these traits (Webb et al., 2016). As previously stated by Langmore (1998) and Amundsen (2000) this phenomenon has largely been ignored until recently and these traits were either regarded as being the consequence of genetic correlation with male ornamentation, functionless, or a result of physiological abnormalities (Lande, 1980; Amundsen, 2000). However, a growing number of studies focused on the evolution of female ornamentation, including plumage and song characteristics (Amundsen et al., 1997; Langmore, 1997, 1998; Amundsen, 2000; Garamszegi et al., 2007; Doutrelant et al., 2008; Mahr et al., 2012; Tobias et al., 2012; Webb et al., 2016).

Several studies have revealed that female choice can be based on several different traits that signal male quality, such as morphological and behavioral traits (Burley, 1981; Johnstone, 1996; Lozano, 2009; Dolnik and Hoi, 2010; Hoi and Griggio, 2012). Multiple traits may (i) act as amplifiers by offering the same information, (ii) have an additive effect whereby the information of several traits complement each other or, (iii) provide different information e.g., about different qualities of the bearer (Burley, 1981; Grafen, 1990; Zuk et al., 1990, 1992; Johnstone, 1995, 1996; Marchetti, 1998; Rivera-Gutierrez et al., 2010). For example bird song and plumage are traits that can signal the same or different information and both traits are driven by sexual selection in males and females (Lande, 1980; Andersson, 1994; Amundsen et al., 1997; Langmore, 1998; Amundsen, 2000; Ball and MacDougall-Shackleton, 2001; Garamszegi et al., 2007; Hegyi et al., 2007, 2008; Cardoso and Hu, 2011). However, the interaction between both traits has hardly been investigated in females (Garamszegi et al., 2007; Webb et al., 2016).

Although, song and plumage traits may carry the same information, these traits can act on different time and spatial scales (Taff et al., 2012). Song usually acts as a long distance signal whereas plumage ornaments act as a short distance signal. When both signals carry the same information, one would predict a positive relationship in the expression of the traits. Alternatively, the expression of both ornamental features might underlie different mechanisms and require different preconditions to maintain an honest signaling function. Furthermore, different production costs may arise, which may consequently represent different qualities. In this case, one would predict that trade-offs between both traits could result in either negative correlations between traits or independent development of different traits, like song and plumage characteristics. However, to our knowledge this trade-off has only been investigated on a phylogenetic scale (Badyaev et al., 2002; Mason et al., 2014; Soma and Garamszegi, 2015).

Whether this also applies to females has, to our knowledge, only been investigated in two comparative studies, focusing on song and plumage development in passerine species. Garamszegi et al. (2007) suggested that singing behavior often occurs in the presence of carotenoid based ornamentation, which is supported by very recent findings from Webb et al. (2016). This positive association might indicate that both traits are generally used in a similar or the same functional context and hence carry the same information content. Nevertheless, only a few case studies examined the interaction between both traits within breeding populations in male songbirds (Møller et al., 1998; Chiver et al., 2008; Taff et al., 2012), and to our knowledge, no study on female birds exists so far. In this study we used the female Superb Fairy-wren (*Malurus cyaneus*) to examine (i) the signaling function of song characteristics and plumage coloration and (ii) the interaction between these two female ornamental features. To determine whether these ornaments reflect female quality we used female body size and body condition as covariates.

The Superb Fairy-wren is an ideal model species to answer our questions because both males and females sing solo chatter songs year-round for territorial defense (Cooney and Cockburn, 1995; Cain and Langmore, 2015), and the structure and complexity of female chatter song is similar to male chatter song (Kleindorfer et al., 2013). Mate attraction may be a secondary function of male chatter song (Dalziell and Cockburn, 2008), but to our knowledge there is no study investigating whether this function applies to female song. In contrast to song, Superb Fairy-wrens have a strong sexual plumage dichromatism. Whereas males have bright blue plumage, females are more cryptic, displaying only an orange lore and a blue tail that reflects in the UV range (own data represented in the Supplementary Material). Maluridae are sensitive to UV and females frequently wave their tail during foraging and social interactions (own unpublished data). This raises the question whether the UV reflecting tail of females is a sexually selected trait (Ödeen et al., 2012).

Previous studies demonstrated a decrease of UV reflectance in worn feathers and from dust accumulation (Örnborg et al., 2002; Zampiga et al., 2004; Griggio et al., 2010, 2011). There is a trade-off between the removal of ectoparasites and dirt from feathers, preening and activities like foraging or increased vigilance against predators (Redpath, 1988; Cucco and Malacarne, 1997; Shawkey et al., 2003; Kapun et al., 2011; Moreno-Rueda and Hoi, 2012). Interestingly there is evidence that similar mechanisms also apply to song features, in particular song rate, which is regarded as a highly variable trait depending on current physiological condition and time of the reproductive cycle (Gil and Gahr, 2002). Therefore, both plumage maintenance and singing behavior force individuals into a trade-off that individuals in poorer condition cannot afford, being forced to invest either more in one or equally, but less in both traits (Andersson et al., 2002).

In many songbird species, song complexity is regarded as stable over the year. The ability to produce complex songs can be an honest signal of quality, because during the development of the neural song system, the expression of neuronal structures and development of the syringeal muscles can be affected by early developmental stress such as under-nourishment (Nowicki et al., 2000; Spencer et al., 2003; Buchanan et al., 2004; Nowicki and Searcy, 2004). Hence, in contrast to plumage characteristics, song complexity can be regarded as less sensitive toward the change of individual condition after the crystallization and determination of singing behavior (Gil and Gahr, 2002).

Our study focuses on the relationship between song complexity and the number of elements females are using (rather than song rate) and UV-reflectance in the blue tail-feathers of female Superb Fairy-wrens. Given both traits may provide different information the question arises whether females use multiple traits to signal quality and condition to male and female conspecifics. Studies investigating relationships between multiple traits within populations are rare and to our knowledge this is the first study focusing on the relationship between features of song, plumage coloration, and morphological traits in a female passerine.

METHODS

Study Sites

The study was carried out during the breeding season between September and November 2012 and 2013 at three study sites on Kangaroo Island: Flinders Chase National Park (35°54′S, 136°47′E), Vivonne Bay Conservation Park (36°00′S, 137°09′E), and Kelly Hill Conservation Park (35°97′S, 136°90′E) and at two study sites on the mainland in South Australia (SA): Cleland Wildlife Conservation Park (35°05′S, 138°41′E) and Newlandhead Conservation Park (35°37′S, 138°29′E). All study sites and territories were chosen on the basis of long term monitoring of Superb Fairy-wren populations, conducted by the BirdLab at Flinders University (Colombelli-Négrel et al., 2010; Kleindorfer et al., 2013).

General Methods

All birds were caught with mist-nets using conspecific playback stimuli and banded with numbered aluminum rings provided by Australian Bird and Bat Banding Scheme (ABBBS) and a unique combination of darvic color rings. Standard measurements of the flattened wing chord length and tail length (to the nearest 0.5 mm) were taken with a ruler, whereas bill length was measured with a caliper (peak to skull, to the nearest 0.1 mm). Body mass was recorded to the nearest 0.1 g.

The research was approved by the Animal Welfare Committee of Flinders University (permit numbers E312 and E386). Permit to undertake scientific research in SA was granted by SA Department of Environment, Water and Natural resources (permit number Z24699-9). All birds were banded under permit number 2601 from the Australian Bird and Bat Banding Scheme.

To assess whether song complexity or plumage coloration is related to female quality, we used female size and body-condition as a determinant of female intrinsic quality and conducted a principal component analyses on these traits (detailed descriptions are attached in the Supplementary Material). Body condition was determined by using residuals of body mass not explained by size (tarsus length; detailed descriptions are attached in the Supplementary Material).

Only fertile females were included in the analyses to control for variation in singing behavior due to reproductive state. Fertility status was verified according to the following three parameters: (i) development of the brood patch (not fully developed), (ii) nest building status (females were considered fertile until the first egg was laid), and (iii) sexual behavior patterns (copulatory behavior, male display, female solicitation behavior; Mulder, 1992 in Cooney and Cockburn, 1995).

Song Recordings and Analyses

Solo songs of color-banded birds occur naturally between 08:00 and 12:00 h (after the dawn chorus) and were recorded from a distance between 5 and 15 m using a parabolic microphone (Telinga Microphones, Sweden) connected to a portable Sound Devices 722 digital audio recorder (Sound Devices LCC, U.S.A). All sound files were recorded as broadcast wave files (24 bit 48 kHz).

Recordings were transcribed to an Apple MacPro (Apple Corporation, U.S.A) and edited with Amadeus Pro 2.1.2 (Hairersoft Inc, Switzerland). Spectrograms were created using Raven 1.5 on the Hann algorithm display type (filter bandwidth 270 Hz, size 256 samples, time grid overlap 50%, grid resolution 2.67 ms, 188 Hz, DFT 256 samples). Only songs that could be confidently assigned to observed color-banded females were analyzed. In total, 82 songs from 28 females were analyzed. For each song, we measured the total number of elements per song (“song length”), and the number of different elements per song (“song complexity”). We define a song as a complex vocalization composed of several different element types (as described by Langmore and Mulder, 1992), and defined an element in the song as a single, continuous trace on a spectrogram. We categorized the different element types according to previously classified element types (A, F, O, P, Q, R, T, U, V, W) developed by Langmore and Mulder (1992), Colombelli-Négrel et al. (2010), Kleindorfer et al. (2013), and Evans and Kleindorfer (2016), and newly identified element types (FL, H, K, L, Z, ZN). For the analysis we determined the element frequency per song as (i) the total number of elements per song (we refer to this variable as “song length”), and (ii) the number of different element types per song (we refer to this variable as “song complexity”).

Spectrometry

We measured the tail coloration of females ($N = 41$), using a JAZ-2000 spectrophotometer and a Xenon-pulsed light source, connected through a bifurcated fiber-optic probe (Ocean Optics, Eerbek, Netherlands). To exclude disturbance by outer light sources and to ensure a standardized distance and angle (90°), a black rubber cylinder was fitted to the top of the probe. Before each measurement the spectrophotometer was recalibrated using a standard white (Avantes, Eerbek, Netherlands); for calibration of black the probe was removed from the light source and the cap of the plug closed (Mahr et al., 2012). Standard descriptors of reflectance spectra were used for quantification of colors. Measurements were taken from five areas on the tail feathers. Calculations were carried out for reflectance in the 320–700 nm range, which is regarded as visual spectrum of most passerine species (Hill and McGraw, 2006). To quantify the UV-reflectance of the blue tail we chose a commonly used variable, namely UV-Chroma (Johnsen et al., 2005; Griggio et al., 2010; Mahr et al., 2012), which is defined as proportion of UV-reflectance on total reflectance (UV:R320–R415/R320–R700; Hill and McGraw, 2006).

Statistical Analyses

To test for the relationships between song length and morphological parameters and plumage characteristics we applied a General Linear Mixed Effects Model (GLMM). Song complexity was analyzed using a Generalized Linear Mixed Effects Model (GZLMM) with a Poisson-distribution as model residuals did not achieve normal distribution even after transformation. Both initial models included UV-Chroma, size, and condition as covariates. As Kangaroo Island and mainland populations are considered to represent different subspecies, we included study site ("location") in all the initial models as a fixed factor to assess local variation in morphology and ornament expression (Dudaniec et al., 2011; Kleindorfer et al., 2013). Also, all the initial models included the interaction between UV-Chroma and location as well as body-size and location because we aimed to test for differences between the populations in regard to UV-Chroma and body-size. Female ID was included as random factor to control for non-independence of multiple measurements from the same female. We had to exclude six females from the analyses since there was not sufficient data available.

The relation between morphological parameters and plumage characteristics was tested separately, due to a difference in the sample size. Analyses were carried out using a General Linear Model (GLM). This GLM included the factor location and the covariates condition, size and the interaction of condition and size. UV-Chroma and condition can show variation during the breeding season, therefore we also included capture month into the GLM. Since the analyses revealed no significant effects of capture date on UV-Chroma and condition, this factor was not included in the GLMM and GZLMM.

We tested for a correlation between song length and complexity using a Spearman's rank correlation test. Song length and complexity were not correlated (Spearman's rank correlation: $\rho = 0.14$, $S = 0.45$, $p = 0.24$), thus we treated these variables independently.

All statistical analyses were performed using "R" (version 2.14.1; R Development Core Team, 2011). We implemented linear mixed effects models using the "lme" function of the "nlme" package. All models were conducted using stepwise forward and backward introduction of terms. Beginning with the interactions, non-significant terms were step by step eliminated from the model. Each eliminated term was re-entered in the final model to obtain statistics (Grafen and Hails, 2002; Engqvist, 2005). In addition to model selection based on *p*-values we performed model averaging using AIC to assess comparability and reliability of both methods. AIC model averaging was implemented using the "model.avg" function of the "MuMIn" package. No differences in the significant results became apparent, and details on the results from model averaging based on AIC-values can be found in the Supplemental Material.

RESULTS

The GLM revealed no significant differences in UV coloration between mainland and island populations [mainland: $N = 18$, island: $N = 23$; $F_{(1,40)} = 2.29$, $p = 0.13$], furthermore no significant effect of month of capture was found on plumage color [$F_{(1,37)} = 0.87$, $p = 0.46$]. Female size was not related to plumage [$F_{(1,39)} = 0.72$, $p = 0.40$], and no relation between UV coloration and female condition was detectable [$F_{(1,39)} = 0.18$, $p = 0.67$]. Also, there were no significant interactions between location and body size [$F_{(1,39)} = 0.15$, $p = 0.70$] or month and condition [$F_{(1,39)} = 0.84$, $p = 0.48$].

Female song length was neither related to her condition [Mainland: $N = 13$, Island: $N = 9$; GLMM: $F_{(1,17)} = 1.93$, $p = 0.18$] nor to her UV-Chroma [GLMM: $F_{(1,19)} = 0.07$, $p = 0.79$]. Also, the interaction of both variables turned out to be non-significant [GLMM: $F_{(1,18)} = 0.06$, $p = 0.95$]. Interestingly though, we found that study site predicted female song length as females from Kangaroo Island produced significantly longer songs compared to females from mainland populations [GLMM: $F_{(1,20)} = 10.79$, $p < 0.01$; **Figure 1**]. Also, we found a significant interaction effect of study site and female size on song length [GLMM: $F_{(1,20)} = 5.66$, $p = 0.03$]: Larger females produce longer songs compared to smaller ones, though this effect is only evident on Kangaroo Island (see **Figure 2**). We found no significant main effect of female size on song length [GLMM: $F_{(1,20)} = 0.81$, $p = 0.38$].

In contrast to song length, song complexity did not differ significantly between study sites (mainland: $N = 13$, island: $N = 9$; GZLMM: $\beta \pm SE = -0.19 \pm 0.11$, $z = -1.69$, $p = 0.09$), though this effect was only marginally non-significant. Female size (GZLMM: $\beta \pm SE = -0.08 \pm 0.05$, $z = 1.58$, $p = 0.11$) and the interaction between female size and study site (GZLMM: $\beta \pm SE = 0.03 \pm 0.13$, $z = 0.21$, $p = 0.83$) turned out to have no significant relationship with song complexity. Also, female condition (GZLMM: $\beta \pm SE = -0.003 \pm 0.01$, $z = -0.26$, $p = 0.79$), UV-Chroma (GZLMM: $\beta \pm SE = -0.80 \pm 1.04$, $z = -0.77$, $p = 0.44$) and the interaction between UV-Chroma and

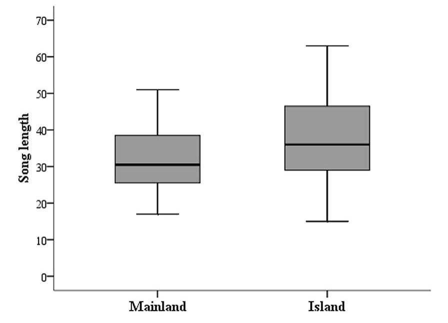

FIGURE 1 | Boxplot of female song length originating from Mainland or Island populations.

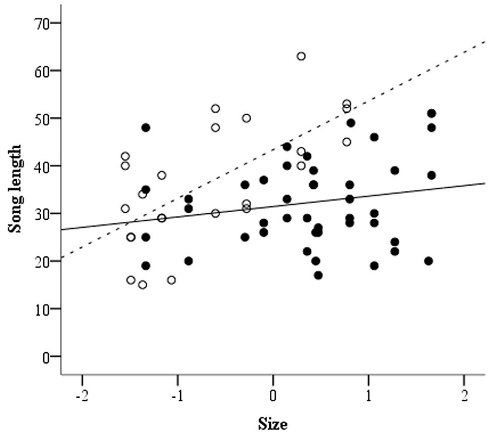

FIGURE 2 | Relationship between female size and song length in mainland (black circles and solid line; $R^2 = 0.04$) and island (white circles and dashed line; $R^2 = 0.44$) populations.

study site (GZLMM: $\beta \pm SE = 1.44 \pm 3.37$, $z = 0.42$, $p = 0.67$) showed no significant relationship with song complexity.

DISCUSSION

Our results show no relationship between plumage ornamentation and song characteristics in female Superb Fairy-wrens, but we revealed a positive relationship between the song length (total number of elements females produce per song) and body-size in females of the Kangaroo Island sub-species. Thus, female Superb Fairy-wrens that sing songs composed of more elements are bigger than females with shorter songs.

In our study populations, the average number of elements varies dramatically between females (between 16 and 50 elements per song). Some females produce more than twice the number of elements than others, which reveals strong individual differences in song strophe length. Consequently for fertile female Superb Fairy-wrens song length may possibly act as a signal for conspecifics to indicate quality. Our results also suggest that songs produced by females of the Kangaroo Island subspecies are significantly longer. These results are in line with previous findings by Kleindorfer et al. (2013), possibly indicating selection processes favoring the production of longer songs within the island population.

Hence, given that size can be an indicator of condition during early development, our results imply that song might act as an honest signal and underlies sexual selection processes in female Superb Fairy-wrens. The idea that female song signals individual quality is supported by earlier studies in Superb Fairy-wrens and New Zealand bellbirds (*Anthornis melanura*), indicating that female song performance (song rate and song complexity) predicts reproductive success (Cain et al., 2015; Brunton et al., 2016).

There are several explanations for why song length in female Superb Fairy-wrens could be an honest signal (Martin-Vivaldi et al., 1998; Farrell et al., 2012; Ferrer et al., 2015). First the production of longer songs is energetically demanding and requires certain physiological preconditions, since it forces females into a trade-off between allocating energy resources toward singing or other activities (Gil and Gahr, 2002). Secondly, the primary function of female song in Superb Fairy-wrens is suggested to be resource defense (Cooney and Cockburn, 1995; Cain and Langmore, 2015). In this context song length might be an indicator for the ability of an individual to defend resources. Finally, very recent findings (Kleindorfer et al., 2016), support the idea, that singing behavior in female Superb Fairy-wrens can also be costly in terms of increased nest-predation. Even though the study by Kleindorfer et al. (2016) refers to song rate rather than song length, one might expect that females producing longer songs may also face higher predation risk by exposing themselves toward predators.

Interestingly, the relationship between size and song length only applies to females from the Kangaroo Island subspecies, but not to females from the mainland populations. However, due to the low sample size, this result has to be treated with caution. A possible explanation for this result could be that Superb Fairy-wrens are in general considered to be long-lived and maintain long-term territories over several years. Stable territories like on the mainland may imply a reduced necessity of intense territorial behavior. In contrast some Superb Fairy-wren populations on Kangaroo Island have been affected by severe bushfires in 2007 (Peace et al., 2011). Within the last years the population started to recover and the number of breeding pairs is increasing in this region. One might assume that individuals face increasing competition from new intruders. Therefore, more competitive individuals, with the ability to maintain larger territories and therefore more resources, should be favored by selection processes. Given that song can be perceived over longer distance and indicates body-size, female song length might signal competitive abilities toward neighbors and intruders (Searcy et al., 2008). Therefore, singing behavior might primarily be of importance to continuously communicate dominance and prevent actual intrusion. Given that, due to natural reestablishment of breeding populations, Kangaroo Island birds might face more frequent encounters with intruders and investment into signals indicating quality might be beneficial to retain breeding sites. Furthermore, this idea is in line with previous findings from Cooney and Cockburn (1995), who demonstrated that female song-rate increased when territories were newly established.

Female song length might also serve as a quality indicator for male conspecifics (Amundsen, 2000). Even though clutch size might not be affected by body size, as females lay a maximum of three eggs per clutch in our population (own observation), choosing bigger females might provide other direct and indirect benefits to males. Size parameters can affect performance in foraging and territorial defense and, as previously mentioned, size can act as indicator for better condition during early development and might signal good genetic quality (Johnson, 1987; Amundsen et al., 1997; Amundsen, 2000). However, given that Superb Fairy-wrens are known to have the highest number of extra pair fertilizations within passerines (Double et al., 1997) and song acts as a signal over long distances, females might also signal quality to possible extra-pair mating partners. The recruitment of extra-pair fertilizations might in turn enhance female reproductive success by increasing genetic variability in the offspring (Andersson, 1994).

Our analyses further reveal that song complexity and song length are not correlated, raising the question of whether both song features signal different quality traits and carry multiple signaling functions. However, song complexity, which has been shown to be an important male song feature for many species (Gil and Gahr, 2002), seems to play only a minor role for female Superb Fairy-wrens. In our study, female song complexity does not reflect female condition or size, nor, in contrast to song length, varies significantly between populations, which has already been shown by previous studies (Dudaniec et al., 2011; Kleindorfer et al., 2013). Furthermore, female song complexity is not related to UV-reflectance of the tail feathers. Also the low variation in song complexity (between three and eight different elements) in comparison to the average number of elements between individual females points toward an inferior role in sexual selection. In previous studies it has been suggested that in some species male repertoire size (e.g., number of elements or songs males produce) does not predict pairing success and therefore plays a minor role in selection processes (Catchpole, 1986; Gil and Gahr, 2002; Byers and Kroodsma, 2009). This might also apply to female song complexity in Superb Fairy-wrens. Nevertheless, it has to be considered that female song complexity reflects quality parameters not recorded in this study.

Whereas a relationship between ornamental features and song performance has been found in interspecific comparisons for female songbirds (Garamszegi et al., 2007; Webb et al., 2016), our results indicate that there is no relationship between UV-Chroma and song features in female Superb Fairy-wrens. This indicates that both traits have evolved independently, rather than co-evolved.

However, it has to be considered that both studies did not examine relationships between song performance and plumage coloration within populations and Garamszegi et al. (2007) focused on carotenoid based plumage features (Garamszegi et al., 2007; Webb et al., 2016). Unlike carotenoid based coloration, UV-coloration is due to melanin based coloration and keratin structure (Prum, 2006). Since these two types of plumage ornaments underlie different physiological mechanisms, they may underlie different selection processes.

UV-Chroma is known to reflect condition of individuals, since the maintenance of UV-reflectance is time consuming and might further result in a trade-off between preening and e.g., parental activities or vigilance behavior (Redpath, 1988; Cucco and Malacarne, 1997; Shawkey et al., 2003). In this context, we expected a correlation between UV-Chroma and condition, but this particular prediction was not supported by our results. One possible explanation might be that the UV-coloration of the plumage is determined by the condition during molt, as shown in male Superb Fairy-wrens (Mulder and Magrath, 1994).

In summary, this is one of the first studies investigating multiple signals in a female songbird, suggesting that plumage features and song performance might underlie different selection processes. Our study revealed that song length is related to a trait reflecting quality and supports the idea of song as a sexually selected trait in female passerines (Cain et al., 2015; Kleindorfer et al., 2016). Our data provides new information on female song and plumage ornaments and more importantly, it extends our understanding of singing behavior in female songbirds.

AUTHOR CONTRIBUTIONS

KM, HH, and MG designed the study; KM and CE conducted the field work and the data collection; KM, HH, KT, and CE performed the statistical analyses of the data; KM, HH, and MG wrote the manuscript with contributions from KT and CE.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2016.00043

REFERENCES

Amundsen, T. (2000). Why are female birds ornamented? *Trends Ecol. Evol.* 15, 149–155. doi: 10.1016/S0169-5347(99)01800-5

Amundsen, T., Forsgren, E., and Hansen, L. T. T. (1997). On the function of female ornaments: male Bluethroats prefer colourful females. *Proc. R. Soc. B* 264, 1579–1586. doi: 10.1098/rspb.1997.0220

Andersson, M. B. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.

Andersson, S., Pryke, S. R., Örnborg, J., Lawes, M. J., and Andersson, M. (2002). Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a Widowbird. *Am. Nat.* 160, 683–691. doi: 10.1086/342817

Badyaev, A. V., Hill, G. E., and Weckwort, B. V. (2002). Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution* 56, 412–419. doi: 10.1111/j.0014-3820.2002.tb01350.x

Ball, G. F., and MacDougall-Shackleton, S. A. (2001). Sex differences in songbirds 25 years later: what have we learned and where do we go? *Microsci. Res. Tech.* 54, 327–334. doi: 10.1002/jemt.1146

Brunton, D. H., Roper, M. M., and Harmer, A. M. T. (2016). Female song rate and structure predict reproductive success in a socially monogamous bird. *Front. Ecol. Evol.* 4:13. doi: 10.3389/fevo.2016.00013

Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A. R., and Catchpole, C. K. (2004). Developmental stress selectively affects the song control nucleus HVC in the Zebra finch. *Proc. R. Soc. Lond. B* 271, 2381–2386. doi: 10.1098/rspb.2004.2874

Burley, N. (1981). Mate choice by multiple criteria in a monogamous species. *Am. Nat.* 117, 515–528. doi: 10.1086/283732

Burley, N. (1986). Sexual selection for aesthetic traits in species with biparental care. *Am. Nat.* 127, 415–445. doi: 10.1086/284493

Byers, B. E., and Kroodsma, D. E. (2009). Female mate choice and songbird song repertoires. *Anim. Behav.* 77, 13–22. doi: 10.1016/j.anbehav.2008.10.003

Cain, K. E., Cockburn, A., and Langmore, N. E. (2015). Female song rates in response to simulated intruder are positively related to reproductive success. *Front. Ecol. Evol.* 3:119. doi: 10.3389/fevo.2015.00119

Cain, K. E., and Langmore, N. E. (2015). Female and male song rates across breeding stage: testing for sexual and nonsexual functions of female song. *Anim. Behav.* 109, 65–71. doi: 10.1016/j.anbehav.2015.07.034

Cardoso, G. C., and Hu, Y. (2011). Birdsong performance and the evolution of simple (rather than elaborate) sexual signals. *Am. Nat.* 178, 679–686. doi: 10.1086/662160

Catchpole, C. K. (1986). Song repertoires and reproductive success in the great reed warbler *Acrocephalus arundinaceus*. *Behav. Ecol. Sociobiol.* 19, 439–445. doi: 10.1007/BF00300547

Chiver, I., Stutchbury, B. J. M., and Morton, E. S. (2008). Do male plumage and song characteristics influence female off-territory forays and paternity in the hooded warbler? *Behav. Ecol. Sociobiol.* 62, 1981–1990. doi: 10.1007/s00265-008-0629-x

Colombelli-Négrel, D., Robertson, J., and Kleindorfer, S. (2010). Nestling presence affects the anti-predator response of adult superb fairy-wrens (*Malurus cyaneus*). *Acta Ethol.* 13, 69–74. doi: 10.1007/s10211-010-0072-7

Cooney, R., and Cockburn, A. (1995). Territorial defence is the major function of female song in the superb fairy-wren, *Malurus cyaneus*. *Anim. Behav.* 49, 1635–1647. doi: 10.1016/0003-3472(95)90086-1

Cucco, M., and Malacarne, G. (1997). The effect of supplemental food on time budget and body condition in the Black Redstart *Phoenicurus ochruros*. *Ardea* 85, 211–221.

Dalziell, A. H., and Cockburn, A. (2008). Dawn song in superb fairy-wrens: a bird that seeks extrapair copulations during the C. *Anim. Behav.* 75, 489–500. doi: 10.1016/j.anbehav.2007.05.014

Dolnik, O., and Hoi, H. (2010). Honest signalling, dominance hierarchies and body condition in House Sparrows *Passer domesticus* (Aves: Passeriformes) during acute coccidiosis. *Biol. J. Linn. Soc.* 99, 718–726. doi: 10.1111/j.1095-8312.2010.01370.x

Double, M. C., Dawson, D., Burke, T., and Cockburn, A. (1997). Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Mol. Ecol.* 6, 691–693. doi: 10.1046/j.1365-294x.1997.00228.x

Doutrelant, C., Gregoire, A., Grnac, N., Gomez, D., Lambrechts, M. M., and Perret, P. (2008). Female coloration indicates female reproductive capacity in blue tits. *J. Evol. Biol.* 21, 226–233. doi: 10.1111/j.14209101.2007.01451.x

Dudaniec, R. Y., Schlotfeldt, B. E., Bertozzi, T., Donnellan, S. C., and Kleindorfer, S. (2011). Genetic and morphological divergence in island and mainland birds: informing conservation priorities. *Biol. Conserv.* 144, 2902–2912. doi: 10.1016/j.biocon.2011.08.007

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim. Behav.* 70, 967–971. doi: 10.1016/j.anbehav.2005.01.016

Evans, C., and Kleindorfer, S. (2016). Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers. *Front. Ecol. Evol.* 4:9. doi: 10.3389/fevo.2016.00009

Farrell, T. M., Weaver, K., An, Y. S., and MacDougall-Shackleton, S. A. (2012). Song bout length is indicative of spatial learning in European starlings. *Behav. Ecol.* 23, 101–111. doi: 10.1093/beheco/arr162

Ferrer, E. S., Garcia-Navas, V., Bueno-Enciso, J., Sanz, J. J., and Ortego, J. (2015). Multiple sexual ornaments signal heterozygosity in male blue tits. *Biol. J. Linn. Soc.* 115, 362–375. doi: 10.1111/bij.12513

Garamszegi, L. Z., Pavlova, D. Z., Eens, M., and Møller, A. P. (2007). The evolution of song in female birds in Europe. *Behav. Ecol.* 18, 86–96. doi: 10.1093/beheco/arl047

Gil, D., and Gahr, M. (2002). The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* 17, 133–141. doi: 10.1016/S0169-5347(02)02410-2

Grafen, A. (1990). Biological signals as handicaps. *J. Theor. Biol.* 144, 517–546. doi: 10.1016/S0022-5193(05)80088-8

Grafen, A., and Hails, R. (2002). *Modern Statistics for the Life Sciences*. Oxford; New York, NY: Oxford University Press.

Grant, P. R. (1998). *Evolution on Islands*. Oxford; New York, NY; Tokyo: Oxford University Press.

Griggio, M., Hoi, H., and Pilastro, A. (2010). Plumage maintenance affects ultraviolet colour and female preference in the Budgerigar. *Behav. Process.* 84, 739–744. doi: 10.1016/j.beproc.2010.05.003

Griggio, M., Serra, L., and Pilastro, A. (2011). The possible effect of dirtiness on structurally based ultraviolet plumage. *Ital. J. Zool.* 78, 90–95. doi: 10.1080/11250003.2010.504238

Hegyi, G., Garamszegi, L. Z., Eens, M., and Török, J. (2008). Female ornamentation and territorial conflicts in collared flycatchers (*Ficedula albicollis*). *Naturwissenschaften* 95, 993–996. doi: 10.1007/s00114-008-0408-6

Hegyi, G., Rosivall, B., Szöllősi, E., Hargitai, R., Eens, M., and Török, J. (2007). A role for female ornamentation in the facultatively polygynous mating system of collared flycatchers. *Behav. Ecol.* 18, 1116–1122. doi: 10.1093/beheco/arm085

Hill, G. E., and McGraw, K. J. (2006). *Bird Coloration - Mechanisms and Measurements*. Cambridge, MA; London: Harvard University Press.

Hoi, H., and Griggio, M. (2008). Dual utility of a melanin-based ornament in Bearded Tits. *Ethology* 114, 1094–1100. doi: 10.1111/j.1439-0310.2008.01566.x

Hoi, H., and Griggio, M. (2012). Bearded reedlings adjust their pair-bond behaviour in relation to the sex and attractiveness of unpaired conspecifics. *PLoS ONE* 7:e32806. doi: 10.1371/journal.pone.0032806

Johnsen, A., Delhey, K., Schlicht, E., Peters, A., and Kempenaers, B. (2005). Male sexual attractiveness and parental effort in blue tits: a test of the differential allocation hypothesis. *Anim. Behav.* 70, 877–888. doi: 10.1016/j.anbehav.2005.01.005

Johnson, K. (1987). Sexual selection in pinyon jays II: male choice and female-female competition. *Anim. Behav.* 36, 1048–1053. doi: 10.1016/S0003-3472(88)80064-2

Johnstone, R. A. (1995). Honest advertisment of multiple qualities using multiple signals. *J. Theor. Biol.* 177, 87–94. doi: 10.1016/S0022-5193(05)80006-2

Johnstone, R. A. (1996). Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Philos. Trans. R. Soc. B* 351, 329–338. doi: 10.1098/rstb.1996.0026

Kapun, M., Darolová, A., Krištofik, J., Mahr, K., and Hoi, H. (2011). Distinct colour morphs in nestling European Bee-Eaters *Merops apiaster*: is there an adaptive value? *J. Ornithol.* 152, 1001–1005. doi: 10.1007/s10336-011-0688-z

Kleindorfer, S., Evans, C., and Mahr, K. (2016). Female in-nest chatter song increases predation. *Biol. Lett.* 12:20150513. doi: 10.1098/rsbl.2015.0513

Kleindorfer, S., Evans, C., Mihailova, M., Colombelli-Négrel, D., Hoi, H., Griggio, M., et al. (2013). When subspecies matter: resident superb fairy-wrens (*Malurus cyaneus*) distinguish the sex and subspecies of intruding birds. *Emu* 113, 259–269. doi: 10.1071/MU12066

Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34, 292–305. doi: 10.2307/2407393

Langmore, N. E. (1997). Song switching in monandrous and polyandrous dunnocks, *Prunella modularis*. *Anim. Behav.* 53, 757–766. doi: 10.1006/anbe.1996.0312

Langmore, N. E. (1998). Functions of duet and solo songs of female birds. *Trends Ecol. Evol.* 13, 136–140. doi: 10.1016/S0169-5347(97)01241-X

Langmore, N. E., and Mulder, R. A. (1992). A novel context for bird song: predator calls prompt male singing in the kleptogamous superb fairy-wren, *Malurus cyaneus*. *Ethology* 90, 143–153. doi: 10.1111/j.1439-0310.1992.tb00828.x

Lozano, G. A. (2009). Multiple cues in mate selection: the sexual interference hypothesis. *Biosci. Hypotheses* 2, 37–42. doi: 10.1016/j.bihy.2008.09.001

Mahr, K., Griggio, M., Granatiero, M., and Hoi, H. (2012). Female attractiveness affects paternal investment: experimental evidence for male differential allocation in blue tits. *Front. Zool.* 9:14. doi: 10.1186/1742-9994-9-14

Marchetti, K. (1998). The evolution of multiple male traits in the yellow-browed leaf warbler. *Anim. Behav.* 55, 361–376. doi: 10.1006/anbe.1997.0586

Martin-Vivaldi, M., Palomino, J. J., and Soler, M. (1998). Song structure in the Hoopoe (*Upupa epops*) - strophe length reflects male condition. *J. Ornithol.* 139, 287–296. doi: 10.1007/BF01653339

Mason, N. A., Shultz, A. J., and Burns, K. J. (2014). Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Proc. R. Soc. B* 281:20140967. doi: 10.1098/rspb.2014.0967

Møller, A. P., Saino, N., Taramino, G., Galeotti, P., and Ferrario, S. (1998). Paternity and multiple signaling: effects of a secondary sexual character and song on paternity in the Barn Swallow. *Am. Nat.* 151, 236–242. doi: 10.1086/286114

Moreno-Rueda, G., and Hoi, H. (2012). Female house sparrows prefer big males with a large white wing bar and fewer feather holes caused by chewing lice. *Behav. Ecol.* 23, 271–277. doi: 10.1093/beheco/arr182

Mulder, R. (1992). *The Evolutionary Ecology of the Mating System of Superb Fairy Wrens*. Ph.D. thesis, Australian National University, Canberra.

Mulder, R. A., and Magrath, M. J. L. (1994). Timing of prenuptial molt as a sexually selected indicator of male quality in superb fairy-wrens (*Malurus cyaneus*). *Behav. Ecol.* 5, 393–400. doi: 10.1093/beheco/5.4.393

Nowicki, S., Hasselquist, D., Bensch, S., and Peters, S. (2000). Nestling growth and song repertoire sire in great reed warblers: evidence for song learning as an indicator mechanism in mate choice. *Proc. R. Soc. Lond. B* 267, 2419–2424. doi: 10.1098/rspb.2000.1300

Nowicki, S., and Searcy, W. A. (2004). Song function and the evolution of female preferences. why birds sing, why brains matter. *Ann. N.Y. Acad. Sci.* 1016, 704–723. doi: 10.1196/annals.1298.012

Ödeen, A., Pruett-Jones, S., Driskell, A. C., Armenta, J. K., and Håstad, O. (2012). Multiple shifts between violet and ultraviolet vision in a family of passerine birds with associated changes in plumage coloration. *Proc. R. Soc. B* 279, 1269–1276. doi: 10.1098/rspb.2011.1777S

Örnborg, J., Andersson, S., Griffith, S. C., and Sheldon, B. C. (2002). Seasonal changes in a ultraviolet structural colour signal in blue tits, *Parus caeruleus*. *Biol. J Linn. Soc.* 76, 237–245. doi: 10.1046/j.1095-8312.2002.00061.x

Peace, M., Mattner, T., and Mills, G. (2011). "The Kangaroo Island bushfires of 2007. A meteorological case study and WRF-fire simulation," in *19th International Congress on Modelling and Simulation* (Perth, WA).

Prum, R. O. (2006). "Anatomy, physics and evolution of avian structure colors," in *Bird Coloration,* eds G. E. Hill and K. J. McGraw (Cambridge: Harvard University Press), 295–353.

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Redpath, S. (1988). Vigilance levels in preening Dunlin *Calidris alpina*. *Ibis* 130, 555–557. doi: 10.1111/j.1474-919X.1988.tb02723.x

Rivera-Gutierrez, H. F., Pinxten, R., and Eens, M. (2010). Multiple signals for multiple messages: great tit, *Parus major*, song signals age and survival. *Anim. Behav.* 80, 451–459. doi: 10.1016/j.anbehav.2010.06.002

Searcy, W. A., Anderson, R. C., and Nowicki, S. (2008). Is bird song a reliable signal of aggressive intent? A reply. *Behav. Ecol. Sociobiol.* 62, 1213–1216. doi: 10.1007/s00265-008-0569-5

Searcy, W. A., and Andersson, M. (1986). Sexual selection and the evolution of song. *Annu. Rev. Ecol. Syst.* 17, 507–533. doi: 10.1146/annurev.es.17.110186.002451

Shawkey, M. D., Estes, A. M., Siefferman, L. M., and Hill, G. E. (2003). Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. *Proc. R. Soc. Lond. B* 270, 1455–1460. doi: 10.1098/rspb.2003.2390

Soma, M., and Garamszegi, L. Z. (2015). Evolution of courtship display in Estrildid finches: dance in relation to female song and plumage ornamentation. *Front. Ecol. Evol.* 3:4. doi: 10.3389/fevo.2015.00004

Spencer, K. A., Buchanan, K. L., Goldsmith, A. R., and Catchpole, C. K. (2003). Song as an honest signal of developmental stress in the Zebra finch (*Taeniopygia guttata*). *Horm. Behav.* 44, 132–139. doi: 10.1016/S0018-506X(03)00124-7

Taff, C. C., Steinberger, D., Clark, C., Belinsky, K., Sacks, H., Freeman-Gallant, C. R., et al. (2012). Multimodal sexual selection in a warbler: plumage and song are related to different fitness components. *Anim. Behav.* 84, 813–821. doi: 10.1016/j.anbehav.2012.07.002

Tobias, J. A., Montgomerie, R., and Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Phil. Trans. R. Soc. B* 367, 2274–2293. doi: 10.1098/rstb.2011.0280

Webb, W. H., Brunton, D. H., Aguirre, D., Thomas, D., Valcu, M., and Dale, J. (2016). Female song occurs in songbirds with more elaborate female coloration and reduced sexual dichromatism. *Front. Ecol. Evol.* 4:13. doi: 10.3389/fevo.2016.00022

Zampiga, E., Hoi, H., and Pilastro, A. (2004). Preening, plumage reflectance and female choice in budgerigars. *Ethol. Ecol. Evol.* 16, 339–349. doi: 10.1080/08927014.2004.9522625

Zuk, M., Ligon, J. D., and Thornhill, R. (1992). Effects of experimental manipulation of male secondary sex characters on female mate preference in red jungle fowl. *Anim. Behav.* 44, 999–1006. doi: 10.1016/S0003-3472(05)80312-4

Zuk, M., Thornhill, R., Ligon, J. D., Johnson, K., Austad, S., Ligon, S. H., et al. (1990). The role of male ornaments and courtship behavior in female mate choice of red jungle fowl. *Am. Nat.* 136, 459–476. doi: 10.1086/285107

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Reference List

Amundsen, T. (2000). Why are female birds ornamented? *Trends in Ecology & Evolution.* 15(4): 149-155.

Andersson, M. (1994). Sexual Selection. Princeton, NJ: Princeton University Press.

Andersson, S., Ornborg, J., & Andersson, M. (1998). Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society of London B: Biological Sciences*. 265(1395): 445-450.

Arnold, A. P. (1992). Developmental plasticity in neural circuits controlling birdsong: sexual differentiation and the neural basis of learning. *Journal of Neurobiology*. 23(10): 1506-1528.

Baker, M. C., & Logue, D. M. (2003). Population differentiation in a complex bird sound: a comparison of three bioacoustical analysis procedures. *Ethology*. 109: 223-242.

Baldassarre, D. T., Greig, E. I., & Webster, M. S. (2016). The couple that sings together stays together: duetting, aggression and extra-pair paternity in a promiscuous bird species. *Biology Letters*. 12(2): 20151025.

Baptista, L. F. (1996) “Nature and its nurturing in avian vocal development”, in Ecology and Evolution of Acoustic Communication in Birds, ed. D. E. Kroodsma & E. H. Miller (Ithaca, NY: Cornell University Press), 39-60.

Baptista, L. F., & Petrinovich, L. (1984). Social interaction, sensitive phases and the song template hypothesis in the white-crowned sparrow. *Animal Behaviour*. 32: 172-81.

Baptista, L. F., & Petrinovich, L. (1986). Song development in the white-crowned sparrow: social factors and sex differences. *Animal Behaviour.* 34: 1359-1371.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J., & Cracraft, J. (2004). Phylogeny and diversification of the largest avian radiation. *Proceedings of the National Academy of Sciences of the United States of America*. 101(30): 11040-11045.

Bateson, P., & Laland, K. N. (2013). Tinbergen's four questions: an appreciation and an update. *Trends in Ecology & Evolution*. 28(12): 712-718.

Beck, N. R., Double, M. C., & Cockburn, A. (2003). Microsatellite evolution at two hypervariable loci revealed by extensive avian pedigrees. *Molecular Biology and Evolution.* 20(1): 54-61.

Beecher, M. D. (1996). “Birdsong learning in the laboratory and field”, in: Ecology and Evolution of Acoustic Communication in Birds, ed D. E. Kroodsma & E. H. Miller (Ithaca, NY: Cornell University Press), 61-78.

Beecher, M. D., & Brenowitz, E. A. (2005). Functional aspects of song learning in songbirds. *Trends in Ecology & Evolution*. 20(3): 143-149.

Beecher, M. D., & Burt, J. M. (2004). The role of social interaction in bird song learning. *Current Directions in Psychological Science*. 13(6): 224-228.

Beecher, M. D., & Campbell, S. E. (2005). The role of unshared songs in singing interactions between neighbouring song sparrows. *Animal Behaviour*. 70(6): 1297-1304.

Beecher, M. D., Campbell, S. E., & Stoddard, P. K. (1994). Correlation of song learning and territory establishment strategies in the song sparrow. *PNAS*. 91: 1450-1454.

Bell, D. A., Trail, P. W., & Baptista, L. F. (1998). Song learning and vocal tradition in Nuttall's white-crowned sparrows. *Animal Behaviour*. 55(4): 939-956.

Bertram, B. (1970). The vocal behaviour of the Indian hill mynah. *Animal Behaviour Monographs.* 3: 81-192.

Blackmore, C. J. (2002). Male song and the dawn chorus of the superb fairy-wren. M.Phil. thesis, Australian National University.

Blumstein, D. T., Cooley, L., Winternitz, J., Daniel, J. C. (2008). Do yellow-bellied marmots respond to predator vocalizations? *Behavioral Ecology and Sociobiology*. 62: 457–468.

Böhner, J. (1983). Song learning in the zebra finch (*Taeniopygia guttata*): selectivity in the choice of a tutor and accuracy of song copies. *Animal Behaviour*. 31(1): 231-237.

Bolhuis, J. J., & Gahr, M. (2006). Neural mechanisms of birdsong memory. *Nature Reviews Neuroscience*. 7(5): 347-357.

Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., et al., (2012). Costs of dispersal. *Biological Reviews*. 87(2): 290-312.

Bortolotti, G. R., & Iko, W. M. (1992). Non-random pairing in American kestrels: mate choice versus intra-sexual competition. *Animal Behaviour.* 44(5): 811-821.

Brainard, M. S., & Doupe, A. J. (2002). What songbirds teach us about learning. *Nature*. 417(6886): 351-358.

Brenowitz, E. A. (1991). Evolution of the vocal control system in the avian brain. *Seminars in Neuroscience*. 3: 399-407.

Brenowitz, E. A., & Arnold, A. P. (1986). Interspecific comparisons of the size of neural song control regions and song complexity in duetting birds: evolutionary implications. *Journal of Neuroscience*. 6: 2875 – 2879.

Brenowitz, E. A., Arnold, A. P., & Levin, R. N. (1985). Neural correlates of female song in tropical duetting birds. *Brain Research*. 343(1): 104-112.

Brenowitz, E. A., & Beecher, M. D. (2005). Song learning in birds: diversity and plasticity, opportunities and challenges. *Trends in Neurosciences*. *28*(3): 127-132.

Brenowitz, E. A., & Kroodsma, D. E. (1996). “The Neuroethology of Birdsong”, in Ecology and Evolution of Acoustic Communication in Birds, ed. D. E. Kroodsma and E. H. Miller (Ithaca, NY: Cornell University Press), 285-304.

Brenowitz, E. A., Margoliash, D., & Nordeen, K. W. (1997). An introduction to birdsong and the avian song system. *Journal of Neurobiology*. 33(5): 495-500.

Brown, E. D., & Farabaugh, S. M. (1991). Song sharing in a group-living songbird, the Australian magpie, *Gymnorhina tibicen*. Part III. Sex specificity and individual specificity of vocal parts in communal chorus and duet songs. *Behaviour*. 118: 244-274.

Brunton, D. H., & Li, X. (2006). The song structure and seasonal patterns of vocal behavior of male and female bellbirds (*Anthornis melanura*). *Journal of Ethology*. 24(1): 17-25.

Brunton, D. H., Roper, M. M., & Harmer, A. M. (2016). Female song rate and structure predict reproductive success in a socially monogamous bird. *Frontiers in Ecology and Evolution*. 4: 13.

Buchanan, K. L., & Catchpole, C. K. (1997). Female choice in the sedge warbler *Acrocephalus schoenobaenus* multiple cues from song and territory quality. *Proceedings of the Royal Society of London B: Biological Sciences*. 264(1381): 521-526.

Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A. R., & Catchpole, C. K. (2004). Developmental stress selectively affects the song control nucleus

HVC in the zebra finch. *Proceedings of the Royal Society of London B: Biological Sciences.* 271(1555): 2381-2386.

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R., & Catchpole, C. K. (2003). Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society of London B: Biological Sciences*. 270(1520): 1149-1156.

Burley, N. (1983). The meaning of assortative mating. *Ethology and Sociobiology*. 4(4): 191-203.

Byers, B. E., & King, D. I. (2000). Singing by female Chestnut-sided Warblers*. The Wilson Bulletin*. 112(4): 547-550.

Byers, B. E., & Kroodsma, D. E. (2009). Female mate choice and songbird song repertoires. *Animal Behaviour*. 77: 13-22.

Cain, K. E., Cockburn, A., & Langmore, N. (2015). Female song rates in response to simulated intruder are positively related to reproductive success. *Frontiers in Ecology and Evolution*. 3:119.

Cain, K. E., & Langmore, N. E. (2015). Female and male song rates across breeding stage: testing for sexual and nonsexual functions of female song. *Animal Behaviour*. 109: 65-71.

Casgrain, P., and Legendre, P. (2001). The R Package for multivariate and spatial analysis, version 4.0 D6 – User's Manual. Departament de sciences biologiques: Université de Montreal, Montreal.

Catchpole, C. K. (1980). Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour.* 74(1): 149-165.

Catchpole, C. K., & Slater, P. J. B. (2008). Bird Song: Biological Themes and Variations. 2nd Edn. Cambridge, UK: Cambridge University Press.

Chan, A. A. Y. H., & Blumstein, D. T. (2011). Attention, noise, and implications for wildlife conservation and management. *Applied Animal Behaviour Science*. 131(1): 1-7.

Charif, R. A., Waack, A. M., & Strickman, L. M. (2010). RavenPro 1.4 User’s Manual. Ithaca, NY: Cornell Laboratory of Ornithology.

Chiver, I., Stutchbury, B. J., & Morton, E. S. (2015). The function of seasonal song in a tropical resident species, the Red-throated Ant-tanager (*Habia fuscicauda*). *Journal of Ornithology.* 156(1): 55-63.

Christensen, R., & Kleindorfer, S. (2007). Assortative pairing and divergent evolution in Darwin’s Small Tree Finch, *Camarhynchus parvulus*. *Journal of Ornithology*. 148(4): 463-470.

Christensen, R., Kleindorfer, S., & Robertson, J. (2006). Song is a reliable signal of bill morphology in Darwin's small tree finch *Camarhynchus parvulus*, and vocal performance predicts male pairing success. *Journal of Avian Biology*, 37(6): 617-624.

Clayton, N. S. (1987). Song tutor choice in zebra finches. *Animal Behaviour*. 35(3): 714-721.

Clayton, N. S. (1988). Song learning and mate choice in estrildid finches raised by two species. *Animal Behaviour*. 36(6): 1589-1600.

Clayton, N., & Pröve, E. (1989). Song discrimination in female zebra finches and Bengalese finches. *Animal Behaviour*. 38(2): 352-354.

Clutton-Brock, T. (2007). Sexual selection in males and females. *Science*. 318(5858): 1882-1885.

Clutton-Brock, T. (2009). Sexual selection in females. *Animal Behaviour.* 77(1): 3-11.

Cockburn, A. (2006). Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society of London B: Biological Sciences*. 273: 1375–1383.

Cockburn, A., Dalziell, A. H., Blackmore, C. J., Double, M. C., Kokko, H., Osmond, H. L., et al., (2009). Superb fairy-wren males aggregate into hidden leks to solicit extragroup fertilizations before dawn. *Behavioral Ecology*. 20(3): 501-510.

Cockburn, A., Osmond, H. L., Mulder, R. A., Green, D. J., & Double, M. C. (2003). Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy‐wren *Malurus cyaneus*. *Journal of Animal Ecology*. 72(2): 189-202.

Cockburn, A., Sims, R. A., Osmond, H. L., Green, D. J., Double, M. C., & Mulder, R. A. (2008). Can we measure the benefits of help in cooperatively breeding birds: the case of superb fairy‐wrens *Malurus cyaneus*?. *Journal of Animal Ecology*. 77(3): 430-438.

Collins, S. A., Hubbard, C., & Houtman, A. M. (1994). Female mate choice in the zebra finch—the effect of male beak colour and male song. *Behavioral Ecology and Sociobiology*. 35(1): 21-25.

Colombelli-Négrel, D. (2016). Female splendid and variegated fairy-wrens display different strategies during territory defence. *Animal Behaviour*. 119: 99-110.

Colombelli-Négrel, D., Hauber, M. E., & Kleindorfer, S. (2014). Prenatal learning in an Australian songbird: habituation and individual discrimination in superb fairy-wren embryos. *Proceedings of the Royal Society of London B: Biological Sciences*. 281(1797): 20141154.

Colombelli-Négrel, D., Hauber, M. E., Robertson, J., Sulloway, F. J., Hoi, H., Griggio, M., & Kleindorfer, S. (2012). Embryonic learning of vocal passwords in superb fairy-wrens reveals intruder cuckoo nestlings. *Current Biology*. 22(22): 2155-2160.

Colombelli-Négrel, D., & Kleindorfer, S. (2009). Nest height, nest concealment, and predator type predict nest predation in superb fairy-wrens (*Malurus cyaneus*). *Ecological Research*. 24(4): 921-928.

Colombelli-Négrel, D., Robertson, J., & Kleindorfer, S. (2011). Risky revelations: Superb Fairy-wrens *Malurus cyaneus* respond more strongly to their mate's alarm song. *Journal of Ornithology.* 12: 127-135.

Colombelli-Négrel, D., Schlotfeldt, B. E., & Kleindorfer, S. (2010). High levels of extra-pair paternity in Superb Fairy-wrens in South Australia despite low frequency of auxiliary males. *Emu*. 109(4): 300-304.

Colombelli-Négrel, D., Webster, M. S., Dowling, J. L., Hauber, M. E., & Kleindorfer, S. (2016). Vocal imitation of mother's calls by begging Red-backed Fairy-wren nestlings increases parental provisioning. *The Auk*. 133(2): 273-285.

Cooney, R., & Cockburn, A. (1995). Territorial defence is the major function of female song in the superb fairy-wren, *Malurus cyaneus*. *Animal Behaviour*. 49(6): 1635-1647.

Creighton, E. (2001). Mate acquisition in the European blackbird and its implications for sexual strategies. *Ethology Ecology & Evolution*. 13(3): 247-260.

Dalziell, A. H., & Cockburn, A. (2008). Dawn song in superb fairy-wrens: a bird that seeks extrapair copulations during the dawn chorus. *Animal Behaviour*. 75(2): 489-500.

Dalziell, A. H., & Welbergen, J. A. (2016). Elaborate mimetic vocal displays by female Superb lyrebirds. *Frontiers in Ecology and Evolution*. 4: 34.

Darwin, C. (1859). The Origin of Species. London: J. Murray.

Darwin, C. (1871). The Descent of Man. London: J. Murray.

Delestrade, A. (2001). Sexual size dimorphism and positive assortative mating in Alpine Choughs (*Pyrrhocorax graculus*). *The Auk*. 118(2): 553-556.

DeWolfe, B. B., Baptista, L. F., & Petrinovich, L. (1989). Song development and territory establishment in Nuttall's White-crowned Sparrows. *Condor*. 397-407.

Double, M., & Cockburn, A. (2000). Pre–dawn infidelity: females control extra-pair mating in superb fairy–wrens. *Proceedings of the Royal Society of London B: Biological Sciences*. 267(1442): 465-470.

Double, M. C., & Cockburn, A. (2003). Subordinate superb fairy-wrens (*Malurus cyaneus*) parasitize the reproductive success of attractive dominant males. *Proceedings of the Royal Society of London B: Biological Sciences*. 270(1513): 379-384.

Double, M. C., Dawson, D., Burke, T., & Cockburn, A. (1997). Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren Malurus cyaneus. Molecular Ecology, 6(7), 691-693.

Double, M. C., Peakall, R., Beck, N. R., & Cockburn, A. (2005). Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution*. 59(3): 625-635.

Dowling, J. L., Colombelli-Négrel, D., & Webster, M. S. (2016). Kin signatures learned in the egg? Red-backed fairy-wren songs are similar to their mother's in-nest calls and songs. *Frontiers in Ecology and Evolution*. 4: 48.

Dowling, J. L., & Webster, M. S. (2013). The form and function of duets and choruses in Red-backed Fairy-wrens. *Emu*. 113(3): 282-293.

Dowling, J., & Webster, M. S. (2016). An experimental test of duet function in a fairy-wren (*Malurus*) with moderate cuckoldry rates. *Behavioral Ecology.* 27(1): 228-236.

Dudaniec, R. Y., Schlotfeldt, B. E., Bertozzi, T., Donnellan, S. C., & Kleindorfer, S. (2011). Genetic and morphological divergence in island and mainland birds: informing conservation priorities. *Biological Conservation.* 144(12): 2902-2912.

Dunn, P. O., and Cockburn, A. (1999). Extrapair mate choice and honest signaling in cooperatively breeding superb fairy-wrens. *Evolution*. 936-946.

Dunn, P. O., Cockburn, A., & Mulder, R. A. (1995). Fairy-wren helpers often care for young to which they are unrelated. *Proceedings of the Royal Society of London B: Biological Sciences*. 259(1356): 339-343.

Eales, L. A. (1985). Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Animal Behaviour*. 33(4): 1293-1300.

Eales, L. A. (1987). Do zebra finch males that have been raised by another species still tend to select a conspecific song tutor? *Animal Behaviour*. 35(5): 1347-1355.

Eberhardt, L. S. (1994). Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *The Auk*. 124-130.

Eens, M., Pinxten, R., & Verheyen, R. F. (1991). Male song as a cue for mate choice in the European starling. *Behaviour*. 116(3): 210-238.

Eens, M., Pinxten, R., & Verheyen, R. F. (1992). No overlap in song repertoire size between yearling and older starlings *Sturnus vulgaris*. *Ibis*. 134(1): 72-76.

Eriksen, A., Lampe, H. M., & Slagsvold, T. (2009). Interspecific cross-fostering affects song acquisition but not mate choice in pied flycatchers, *Ficedula hypoleuca*. *Animal Behaviour*. 78(4): 857-863.

Espmark, Y. O., & Lampe, H. M. (1993). Variations in the song of the pied flycatcher within and between breeding seasons. *Bioacoustics*. 5(1-2): 33-65.

Evans, C., & Kleindorfer, S. (2016). Superb fairy-wren (*Malurus cyaneus*) sons and daughters acquire song elements of mothers and social fathers. *Frontiers in Ecology and Evolution*. 4: 9.

Farabaugh, S. M., Brown, E. D., & Hughes, M. (1992). Cooperative territorial defense in the Australian Magpie, *Gymnorhim tibicen* (Passeriformes, Cracticidae), a group-living songbird. *Ethology*. 92: 283-292.

Fontaine, J. J., & Martin, T. E. (2006). Habitat selection responses of parents to offspring predation risk: an experimental test. *The American Naturalist*. 168(6): 811-818.

Gahr, M., Metzdorf, R., Schmidl, D., & Wickler, W. (2008). Bi-directional sexual dimorphisms of the song control nucleus HVC in a songbird with unison song. *PLoS One*. 3(8): e3073.

Gahr, M., Sonnenschein, E., & Wickler, W. (1998). Sex difference in the size of the neural song control regions in a duetting songbird with similar song repertoire size of males and females. *The Journal of Neuroscience*. *18*(3): 1124-1131.

Garamszegi, L. Z., Eens, M., Erritzøe, J., & Møller, A. P. (2005). Sexually size dimorphic brains and song complexity in passerine birds. *Behavioral Ecology*. 16(2): 335-345.

Garamszegi, L. Z., Pavlova, D. Z., Eens, M., & Møller, A. P. (2007). The evolution of song in female birds in Europe. *Behavioral Ecology*. 18(1): 86-96.

Geberzahn, N., & Gahr, M. (2013). Song learning in male and female *Uraeginthus cyanocephalus*, a tropical songbird species. *Journal of Comparative Psychology*. 127(4): 352.

Gil, D., Cobb, J. L., & Slater, P. J. (2001). Song characteristics are age dependent in the willow warbler, *Phylloscopus trochilus*. *Animal Behaviour*. 62(4): 689-694.

Gil, D., & Gahr, M. (2002). The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution*. 17(3): 133-141.

Gil, D., Naguib, M., Riebel, K., Rutstein, A., & Gahr, M. (2006). Early condition, song learning, and the volume of song brain nuclei in the zebra finch (*Taeniopygia guttata*). *Journal of Neurobiology*. 1602-1612.

Gowaty, P. A. (1997). "Sexual dialectics, sexual selection, and variation in reproductive behaviour", in Feminism and Evolutionary Biology (Springer US), 351-384.

Gowaty, P. A. (2003). Sexual natures: How feminism changed evolutionary biology. *Signs*. 28(3): 901-921.

Grant, P. R. (1991). Natural selection and Darwin's finches. *Scientific American*. 265(4): 82-87.

Grant, B. R., & Grant, P. R. (1996). Cultural inheritance of song and its role in the evolution of Darwin's finches. *Evolution*. 2471-2487.

Greig, E. I., Price, J. J., & Pruett-Jones, S. (2013). Song evolution in Maluridae: influences of natural and sexual selection on acoustic structure. *Emu*. 113(3): 270-281.

Greig, E., & Pruett-Jones, S. (2008). Splendid songs: the vocal behaviour of Splendid Fairy-wrens (*Malurus splendens melanotus*). *Emu.* 108(2): 103-114.

Greig, E. I., & Pruett-Jones, S. (2009). A predator-elicited song in the splendid fairy-wren: warning signal or intraspecific display? *Animal Behaviour*. 78(1): 45-52.

Greig, E. I., Taft, B. N., & Pruett-Jones, S. (2012). Sons learn songs from their social fathers in a cooperatively breeding bird. *Proceedings of the Royal Society of London B: Biological Sciences*. rspb20112582.

Griffiths, R., Double, M. C., Orr, K., &Dawson, R. J. (1998). A DNA test to sex most birds. *Molecular Ecology*. 7: 1071-1075.

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour*. 60(5): 667-677.

Hall, M. L. (2004). A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology*. 55(5): 415-430.

Hall, M. L. (2006). Convergent vocal strategies of males and females are consistent with a cooperative function of duetting in Australian magpie-larks. *Behaviour*. 143(4): 425-449.

Hall, M. L., & Peters, A. (2008). Coordination between the sexes for territorial defence in a duetting fairy-wren. *Animal Behaviour*. 76(1): 65-73.

Hall, M. L., Rittenbach, M. R., & Vehrencamp, S. L. (2015). Female song and vocal interactions with males in a neotropical wren. *Frontiers in Ecology and Evolution*. 3: 12.

Hall, Z. J., MacDougall-Shackleton, S. A., Osorio-Beristain, M., & Murphy, T. G. (2010). Male bias in the song control system despite female bias in song rate in streak-backed orioles (*Icterus pustulatus*). *Brain Behaviour and. Evolution*. 76: 168-175.

Hasselquist, D., Bensch, S., & von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*. 381(6579): 229-232.

Hausberger, M., Richard-Yris, M. A., Henry, L., Lepage, L., & Schmidt, I. (1995). Song sharing reflects the social organization in a captive group of European starlings (*Sturnus vulgaris*). *Journal of Comparative Psychology.* 109: 222-241.

Hiebert, S. M., Stoddard, P. K., & Arcese, P. (1989). Repertoire size, territory acquisition and reproductive success in the song sparrow. *Animal Behaviour*. 37: 266-273.

Hile, A. G., Burley, N. T., Coopersmith, C. B., Foster, V. S., & Striedter, G. F. (2005). Effects of male vocal learning on female behavior in the budgerigar, *Melopsittacus undulatus*. *Ethology*. 111(10): 901-923.

Houtman, A. M., & Falls, J. B. (1994). Negative assortative mating in the white-throated sparrow, *Zonotrichia albicollis*: the role of mate choice and intra-sexual competition. *Animal Behaviour*. 48(2): 377-383.

Hrdy, S. B. (1997). Raising Darwin's consciousness. *Human Nature*. 8(1): 1-49.

Hultsch, H., Mundry, R., and Todt, D. (1999) "Learning, representation and retrieval of rule-related knowledge in the song system of birds," in Learning, ed. A. D. Friederici and R. Menzel (Berlin, DE: de Gruyter), 89-111.

Hultsch, H., & Todt, D. (1992). The serial order effect in the song acquisition of birds: relevance of exposure frequency to song models. *Animal Behaviour*. 44: 590-592.

Illes, A. E. (2015). Context of female bias in song repertoire size, singing effort, and singing independence in a cooperatively breeding songbird. *Behavioural Ecology and Sociobiology*. 69: 139-150.

Illes, A. E., & Yunes-Jimenez, L. (2009). A female songbird out-sings male conspecifics during simulated territorial intrusions. *Proceedings of the Royal Society of London B: Biological Sciences*. 276(1658): 981-986.

Immelmann, K. (1969). Song development in the zebra finch and other estrildid finches. *Bird Vocalizations*. 61.

Jawor, J. M., Linville, S. U., Beall, S. M., & Breitwisch, R. (2003). Assortative mating by multiple ornaments in northern cardinals (*Cardinalis cardinalis*). *Behavioral Ecology*. 14(4): 515-520.

Jawor, J. M., & MacDougall-Shackleton, S. A. (2008). Seasonal and sex-related variation in song control nuclei in a species with near-monomorphic song, the northern cardinal. *Neuroscience Letters*. 443(3): 169-173.

Johannessen, L. E., Slagsvold, T., & Hansen, B. T. (2006). Effects of social rearing conditions on song structure and repertoire size: experimental evidence from the field. *Animal Behaviour*. 72(1): 83-95.

Johnstone, R. A., Reynolds, J. D., & Deutsch, J. C. (1996). Mutual mate choice and sex differences in choosiness. *Evolution*. 1382-1391.

Keen, S., Meliza, D. C., Pilowsky, J., & Rubenstein, D. R. (2016). Song in a social and sexual context: vocalizations signal identity and rank in both sexes of a cooperative breeder. *Frontiers in Ecology and Evolution.* 4: 46.

Kempenaers, B., Verheyen, G. R., & Dhondi, A. A. (1997). Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*. 8(5): 481-492.

Kleindorfer, S., Evans, C., Colombelli-Négrel, D., Robertson, J., Griggio, M., & Hoi, H. (2013a). Host response to cuckoo song is predicted by the future risk of brood parasitism. *Frontiers in Zoology*. 10(1): 1.

Kleindorfer, S., Evans, C., Mihailova, M., Colombelli-Négrel, D., Hoi, H., Griggio, M., et al. (2013b). When subspecies matter resident Superb Fairy-wrens (*Malurus cyaneus*) distinguish the sex and subspecies of intruding birds. *Emu.* 113: 259-269.

Kleindorfer, S., Evans, C., & Colombelli-Négrel, D. (2014a). Females that experience threat are better teachers. *Biology Letters.* 10(5): 20140046.

Kleindorfer, S., Hoi, H., Evans, C., Mahr, K., Robertson, J., Hauber, M. E., & Colombelli-Négrel, D. (2014b). The cost of teaching embryos in superb fairy-wrens. *Behavioral Ecology*. 25(5): 1131-1135.

Kleindorfer, S., Evans, C., & Mahr, K. (2016). Female in-nest chatter song increases predation. *Biology Letters.* 12(1): 20150513.

Komdeur, J., Oorebeek, M., van Overveld, T., & Cuthill, I. C. (2005). Mutual ornamentation, age, and reproductive performance in the European starling. *Behavioral Ecology*. 16(4): 805-817.

Konishi, M. (1985). Birdsong: from behavior to neuron. *Annual Review of Neuroscience*. 8(1): 125-170.

Kroodsma, D. E. (1974). Song learning, dialects, and dispersal in the Bewick's Wren. *Zeitschrift für Tierpsychologie*. 35(4): 352-380.

Kroodsma, D. E. (1976). Reproductive development in a female songbird: differential stimulation by quality of male song. *Science*. 192: 574-575.

Kroodsma, D. E., & Pickert, R. (1984). Sensitive phases for song learning: effects of social interaction and individual variation. *Animal Behaviour*. 32(2): 389-394.

Lampe, H. M., & Espmark, Y. O. (1994). Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. *Animal Behaviour*. 47(4): 869-876.

Lampe, H. M., & Saetre, G. P. (1995). Female pied flycatchers prefer males with larger song repertoires. *Proceedings of the Royal Society of London B: Biological Sciences*. 262(1364): 163-167.

Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*. 292-305.

Langmore, N. E. (1998). Functions of duet and solo songs of female birds. *Trends in Ecology and Evolution.* 13: 136-140.

Langmore, N. E., Davies, N. B., Hatchwell, B. J., & Hartley, I. R. (1996). Female song attracts males in the alpine accentor *Prunella collaris*. *Proceedings of the Royal Society of London B: Biological Sciences*. 263(1367): 141-146.

Langmore, N. E., & Mulder, R. A. (1992). A novel context for bird song: predator calls prompt male singing in the kleptogamous superb fairy‐wren, *Malurus cyaneus. Ethology.* 90(2): 143-153.

Law, E., and Ahn, L. V. (2011). Human computation. *Synthesis Lectures on Artificial Intelligence and Machine Learning*. 5: 1-121.

Levin, R. N. (1996). Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus*: I. Removal experiments. *Animal Behaviour*. 52(6): 1093-1106.

Lobato, M., Vellema, M., Gahr, C., Leitão, A., de Lima, S. M. A., Geberzahn, N., & Gahr, M. (2015). Mismatch in sexual dimorphism of developing song and song control system in blue-capped cordon-bleus, a songbird species with singing females and males. *Frontiers in Ecology and Evolution*. 3: 117.

MacDougall-Shackleton, S. A., & Ball, G. F. (1999). Comparative studies of sex differences in the song-control system of songbirds. *Trends in Neuroscience*. 22: 432-436.

Magrath, R. D., Haff, T. M., Horn, A. G., & Leonard, M. L. (2010). Calling in the face of danger: predation risk and acoustic communication by parent birds and their offspring. *Advances in the Study of Behavior*. 41: 187-253.

Mahr, K., Evans, C., Thonhauser, K. E., Griggio, M., & Hoi, H. (2016). Multiple ornaments—multiple signalling functions? The importance of song and UV plumage coloration in female superb fairy-wrens (*Malurus cyaneus*). *Frontiers in Ecology and Evolution.* 4: 43.

Mann, N. I., & Slater, P. J. B. (1995). Song tutor choice by zebra finches in aviaries. *Animal Behaviour.* 49(3): 811-820.

Marler, P. (1970). A comparative approach to vocal learning: song development in White-crowned Sparrows. *Journal of Comparative and Physiological Psychology*. 71(2): 1.

Marler, P., & Peters, S. (1981). Sparrows learn adult song and more from memory. *Science*. 213(4509): 780-782.

Marler, P., & Peters, S. (1982). Long-term storage of learned birdsongs prior to production. *Animal Behaviour*. 30(2): 479-482.

Marler, P., & Peters, S. (1988). The role of song phonology and syntax in vocal learning preferences in the song sparrow, *Melospiza melodia*. *Ethology*. 77(2): 125-149.

Marler, P., and Slabbekoorn, H. (2004). Nature’s Music: The science of birdsong. San Diego: Elsevier Academic Press.

Marshall–Ball, L., & Slater, P. J. (2004). Duet singing and repertoire use in threat signalling of individuals and pairs. *Proceedings of the Royal Society of London B: Biological Sciences*. 271: S440-S443.

Marshall‐Ball, L., & Slater, P. J. (2008). Repertoire sharing by the individual and the pair: Insights into duet function and development in the plain wren *Thryothorus modestus*. *Journal of Avian Biology*. 39(3): 293-299.

Marshall, T. C, Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*. 7: 639-655.

Martin, T. E. (1996). Life history evolution in tropical and south temperate birds: what do we really know? *Journal of Avian Biology*. 27(4): 263-272.

McDonald, P. G., & Wright, J. (2011). Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proceedings of the Royal Society of London B: Biological Sciences*. 278(1723): 3403-3411.

McGregor, P. K., & Krebs, J. R. (1989). Song learning in adult great tits (Parus major): effects of neighbours. *Behaviour.* 108(1): 139-159.

McGregor, P. K., Krebs, J. R., & Perrins, C. M. (1981). Song repertoires and lifetime reproductive success in the great tit (*Parus major*). *American Naturalist*. 149-159.

Medicus, G. (2015). Being Human–Bridging the Gap between the Sciences of Body and Mind. Berlin: VWB 2015. ISBN 978-3-86135-584-7. Translated from German to English by Norbert Hohl and Sonia Kleindorfer

Møller, A. P., Nielsen, J. T., & Garamszegi, L. Z. (2006). Song post exposure, song features, and predation risk. *Behavioral Ecology.* 17(2): 155-163.

Møller, A. P., Nielsen, J. T., & Garamzegi, L. Z. (2008). Risk taking by singing males. *Behavioral Ecology*. 19(1): 41-53.

Moravec, M. L., Striedter, G. F., & Burley, N. T. (2006). Assortative pairing based on contact call similarity in budgerigars, *Melopsittacus undulatus*. *Ethology*. 112(11): 1108-1116.

Morton, E. S. (1996). "A comparison of vocal behaviour among tropical and temperate passerine birds", in Ecology and Evolution of Acoustic Communication in Birds, ed. D. E. Kroodsma and E. H. Miller (Ithaca, NY: Cornell University Press), 258-268.

Mountjoy, J. D., & Lemon, R. E. (1995). Extended song learning in wild European starlings. *Animal Behaviour*. 49(2): 357-366.

Mountjoy, D. J., & Lemon, R. E. (1996). Female choice for complex song in the European starling: a field experiment. *Behavioral Ecology and Sociobiology*. 38(1): 65-71.

Mulder, R. A. (1995). Natal and breeding dispersal in a co-operative, extra-group-mating bird. *Journal of Avian Biology*. 234-240.

Mulder, R. A. (1997). Extra-group courtship displays and other reproductive tactics of superb fairy-wrens. *Australian Journal of Zoology*. 45: 131-143.

Mulder, R. A., Dunn, P. O., Cockburn, A., Lazenby-Cohen, K. A., & Howell, M. J. (1994). Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proceedings of the Royal Society of London B: Biological Sciences*. 255(1344): 223-229.

Mulder, R. A., & Langmore, N. E. (1993). Dominant males punish helpers for temporary defection in superb fairy-wrens. *Animal Behaviour*. 45(4): 830-833.

Mulder, R. A., & Magrath, M. J. (1994). Timing of prenuptial molt as a sexually selected indicator of male quality in superb fairy-wrens (*Malurus cyaneus*). *Behavioral Ecology*. *5*(4): 393-400.

Najar, N., & Benedict, L. (2015). Female song in new world wood-warblers (Parulidae). *Frontiers in Ecology and Evolution.* 3: 139.

Nelson, D. A. (1997). “Social interaction and sensitive phases for song learning”, in Social influences on vocal development, ed. C. T. Snowdon and M. Hausberger (Cambridge, UK: Cambridge UP), 7-22.

Neubauer, R. L. (1999). Super-normal length song preferences of female zebra finches (*Taeniopygia guttata*) and a theory of the evolution of bird song. *Evolutionary Ecology*. 13(4): 365-380.

Nottebohm, F., & Arnold, A. P. (1976). Sexual dimorphism in vocal control areas of the songbird brain. *Science*. 194(4261): 211-213.

Nottebohm, F., & Nottebohm, M. E. (1978). Relationship between song repertoire and age in the canary, *Serinus canarius*. *Zeitschrift für Tierpsychologie*. 46(3): 298-305.

Nowicki, S., Searcy, W., & Peters, S. (2002). Brain development, song learning and mate choice in birds: a review and experimental test of the “nutritional stress hypothesis". *Journal of Comparative Physiology A*. 188(11-12): 1003-1014.

Odom, K. J., Hall, M. L., Riebel, K., Omland, K. E., & Langmore, N. E. (2014). Female song is widespread and ancestral in songbirds. *Nature Communications*. 5.

Pasinelli, G., Schiegg, K., & Walters, J. R. (2004). Genetic and environmental influences on natal dispersal distance in a resident bird species. *The American Naturalist*. 164(5): 660-669.

Pavlova, D. Z., Pinxten, R., & Eens, M. (2010). Age-related changes of song traits in female European Starlings (Sturnus vulgaris). *Animal Biology.* 60(1): 43-59.

Payne, R. B. (1981). Song learning and social interaction in indigo buntings. *Animal Behaviour.* 29(3): 688-697.

Payne, R. B., & Payne, L. L. (1993). Song copying and cultural transmission in indigo buntings. *Animal Behaviour*. 46(6): 1045-1065.

Peakall, R. & Smouse, P.E. 2006 GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes.* 6: 288-295.

Peters, A., Kingma, S. A. & Delhey, K. (2013). Seasonal male plumage as a multi-component sexual signal: insights and opportunities. *Emu*. 113: 232–247.

Peters, S., Marler, P., & Nowicki, S. (1992). Song sparrows learn from limited exposure to song models. *The Condor*. 94(4): 1016-1019.

Petrinovich, L. (1985). Factors influencing song development in the white-crowned sparrow (*Zonotrichia leucophrys*). *Journal of Comparative Psychology*. 99(1): 15.

Pfenning, A. R., Hara, E., Whitney, O., Rivas, M. V., Wang, R., Roulhac, P. L., Howard, J. T., Wirthlin, M., Lovell, P. V., Ganapathy, G., Mountcastle, J., Moseley, M. A., Thompson, W., Soderblom, E. J., Iriki, A., Kato, M., Gilbert, M. T. P., Zhang, G., Bakken, T., Bongaarts, A., Bernard, A., Lein, E., Mello, C. V., Hartemink, A. J., & Jarvis, E. D. (2014). Convergent transcriptional specializations in the brains of humans and song-learning birds. *Science*. 346: 1256846.

Pilowsky, J. A., & Rubenstein, D. R. (2013). Social context and the lack of sexual dimorphism in song in an avian cooperative breeder. *Animal Behaviour*. 85(4): 709-714.

Potti, J. (2000). Causes and consequences of age-assortative pairing in pied flycatchers (*Ficedula hypoleuca*). *Etología*. 8: 29-36.

Price, J. J. (1998). Family-and sex-specific vocal traditions in a cooperatively breeding songbird. *Proceedings of the Royal Society of London B: Biological Sciences*. 265: 497-502.

Price, J. J. (2009). Evolution and life-history correlates of female song in the New World blackbirds. *Behavioral Ecology*. 20: 967-977.

Price, J. J. (2015). Rethinking our assumptions about the evolution of bird song and other sexually dimorphic signals. *Frontiers in Ecology and Evolution*. 3:40.

Price, J. J., Lanyon, S. M., & Omland, K. E. (2009). Losses of female song with changes from tropical to temperate breeding in the New World blackbirds. *Proceedings of the Royal Society of London B: Biological Sciences*. 276(1664): 1971-1980.

Price, P. H. (1979). Developmental determinants of structure in zebra finch song. *Journal of Comparative and Physiological Psychology.* 93(2): 260.

Raymond, M., & Rousset, F. (1995). Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*. 86(3): 248-249.

Ridley, A. R., & Ashton, B. J. (2015). The benefits of an evolutionary framework for the investigation of teaching behaviour: Emphasis should be taken off humans as a benchmark. *Behavioral and Brain Sciences*. 38.

Riebel, K. (2003). The “mute” sex revisited: vocal production and perception learning in female songbirds. *Advances in the Study of Behavior*. 33: 49-86.

Riebel, K. (2016). Understanding sex differences in form and function of bird song: The importance of studying song learning processes. *Frontiers in Ecology and Evolution*. 4: 62.

Riebel, K., Hall, M.L., & Langmore, N.E. (2005). Female songbirds still struggling to be heard. *Trends in Ecology and Evolution.* 20: 419-420.

Robinson, A. (1949). The biological significance of bird song in Australia. *Emu.* 48(4): 291-315.

Rogers, A. C., Langmore, N. E., & Mulder, R. A. (2007). Function of pair duets in the eastern whipbird: cooperative defense or sexual conflict? *Behavioral Ecology*. 18(1): 182-188.

Roper, A., & Zann, R. (2006). The onset of song learning and song tutor selection in fledgling zebra finches. *Ethology*. 112(5): 458-470.

Rowley, I. C. R. (1965). The life history of the superb blue wren, *Malurus cyaneus*. *Emu*. 64(4): 251-297.

Rowley, I. C. R., & Russell, E. (1997). Fairy-wrens and Grasswrens: Maluridae. Oxford, UK: Oxford University Press.

Rowley, I., & Russell, E. (2007) “Family Maluridae (fairy-wrens)”, in Picathartes to tits and chickadees, Handbook of the Birds of the World, vol. 12, ed J. del. Hoyo, A. Elliott and D. A. Christie (Barcelona: Lynx Edicions), 490–531.

Russell, E. M. (2000). Avian life histories: Is extended parental care the southern secret? *Emu*. 100(5): 377-399.

Russell, E. M., Yom-Tov, Y., & Geffen, E. (2004). Extended parental care and delayed dispersal: northern, tropical, and southern passerines compared. *Behavioral Ecology*. 15(5): 831-838.

Schmidt, K. L., Moore, S. D., MacDougall-Shackleton, E. A., and MacDougall-Shackleton, S. A. (2013). Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. *Animal Behaviour*. 86: 25-35.

Schwabl, H., Dowling, J., Baldassarre, D. T., Gahr, M., Lindsay, W. R., & Webster, M. S. (2015). Variation in song system anatomy and androgen levels does not correspond to song characteristics in a tropical songbird. *Animal Behaviour*. 104: 39-50.

Searcy, W. A., McArthur, P. D., & Yasukawa, K. (1985). Song repertoire size and male quality in song sparrows. *The Condor*. 222-228.

Searcy, W. A., & Yasukawa, K. (1996). “Song and female choice”, in Ecology and Evolution of Acoustic Communication in Birds, ed. D. E. Kroodsma and E. H. Miller (Ithaca, NY: Cornell University Press), 454-472.

Sharp, S. P., & Hatchwell, B. J. (2005). Individuality in the contact calls of cooperatively breeding long-tailed tits (*Aegithalos caudatus*). *Behaviour*. 142(11-12): 1559-1575.

Sharp, S. P., & Hatchwell, B. J. (2006). Development of family specific contact calls in the Long‐tailed Tit *Aegithalos caudatus*. *Ibis*. 148(4): 649-656.

Sharp, S. P., McGowan, A., Wood, M. J., & Hatchwell, B. J. (2005). Learned kin recognition cues in a social bird. *Nature*. 434(7037): 1127-1130.

Slater, P. J., & Mann, N. I. (2004). Why do the females of many bird species sing in the tropics? *Journal of Avian Biology*. 35(4): 289-294.

Smith, L. M., & Burgoyne, L. A. (2004). Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology*. 4(1): 4.

Spencer, K. A., Buchanan, K. L., Goldsmith, A. R., & Catchpole, C. K. (2003). Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Hormones and Behavior*. 44(2): 132-139.

Spencer, K. A., Wimpenny, J. H., Buchanan, K. L., Lovell, P. G., Goldsmith, A. R., & Catchpole, C. K. (2005). Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). *Behavioural Ecology and Sociobiology.* 58: 423-428.

Thorpe, W. H. (1958). The learning of song patterns by birds, with especial reference to the song of the chaffinch *Fringilla coelebs*. *Ibis*. 100(4): 535-570.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift für Tierpsychologie*. 20(4): 410-433.

Tobias, J. A., Montgomerie, R., & Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B: Biology Sciences.* 367(1600): 2274-2293.

Tobias, J. A., Sheard, C., Seddon, N., Meade, A., Cotton, A. J., Nakagawa, S., & Datasets, E. (2016). Territoriality, social bonds, and the evolution of communal signalling in birds. *Frontiers in Ecology and Evolution.* 4: 74.

Todt, D., Hultsch, H., & Heike, D. (1979). Conditions affecting song acquisition in nightingales (*Luscinia megarhynchos L.*). *Zeitschrift für Tierpsychologie*.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*. 4(3): 535-538.

Webb, W. H., Brunton, D. H., Aguirre, J. D., Thomas, D. B., Valcu, M., & Dale, J. (2016). Female song occurs in songbirds with more elaborate female coloration and reduced sexual dichromatism. *Frontiers in Ecology and Evolution*. 4: 22.

Webster, M. S., Tarvin, K. A., Tuttle, E. M., & Pruett-Jones, S. (2004). Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. *Behavioral Ecology*. 15(6): 907-915.

West-Eberhard, M. J. (1979). Sexual selection, social competition, and evolution. *Proceedings of the American Philosophical Society*. 123(4): 222-234.

West-Eberhard, M. J. (1983). Sexual selection, social competition, and speciation. *Quarterly Review of Biology*. 155-183.

Wickler, W., & Sonnenschein, E. (1989). Ontogeny of song in captive duet-signing slate-coloured boubous (*Laniarius fumebris*). A study in birdsong epigenesis. *Behaviour*. 111: 220-233.

Williams, H. (1990). Models for song learning in the zebra finch: fathers or others? *Animal Behaviour*. 39: 745-757.

Wilson, P. L., Towner, M. C., & Vehrencamp, S. L. (2000). Survival and song-type sharing in a sedentary subspecies of the song sparrow. *The Condor*. 102(2): 355-363.

Yamaguchi, A. (2001). Sex differences in vocal learning in birds. *Nature*. 411(6835): 257-258.

Zeh, D. W., & Zeh, J. A. (1988). Condition-dependent sex ornaments and field tests of sexual-selection theory. *The American Naturalist*. 132(3): 454-459.

Zoratto, F., Manzari, L., Oddi, L., Pinxten, R., Eens, M., Santucci, D., et al. (2014). Behavioural response of European starlings exposed to video playback of conspecific flocks: effect of social context and predator threat. *Behavioural Processes*. 103: 269-277.