The function of female and male ornaments in the Lovely fairy-wren

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THESIS ABSTRACT

Ornaments like plumage colours or complex song are generally regarded as male traits that are shaped by sexual selection. By contrast, the factors that shape female elaborate traits have often been overlooked, though they are expressed in females across many taxa. Understanding how trade-offs and selective pressures shape female ornamentation is crucial for advancing our understanding of trait evolution. In this thesis, I investigate the form and function of female and male plumage colour and song in the Lovely fairy-wren (*Malurus amabilis*), a tropical species in which females and males are both highly colourful and vocal. This was investigated over three consecutive years and field seasons in Far North Queensland, Australia.

My thesis research employed field observations, behavioural experiments, and genetic analysis, to test the adaptive function(s) and mechanisms for the evolution of female and male ornamental traits. I explicitly contrast females and males so that we can address, in the light of the abundant work done on males, how females may or may not differ from males.

To provide context for the ornamental traits that are exhibited by this species, I first provide a comprehensive overview of the ecology and breeding biology of the Lovely fairy-wren, since a detailed description on the species natural history prior to this work was lacking.

To understand the function of plumage colouration, I studied whether plumage colour in females and males is a signal and experimentally tested if it functions in a competitive context. Additionally, I assessed whether plumage colour is sexually selected, by examining its signalling content, costs (survival), and its relationship with reproductive and paternity success.

Lastly, I investigated the song function, by describing female and male song structure and examining sex-specific variation in song rate across different contexts. I also used experimental data to examine female and male responses to simulated territorial intrusion.

Overall this thesis provides insight into the form and function of both female and male plumage colours and song. First, it shows that visual and acoustic ornaments are important signalling components in different contexts, suggesting that female ornaments are not just a correlated genetic by-product of traits in males, and that selection favours female (and male) expression of traits. Second, the information conveyed by plumage colouration seems to be context-dependent in relation to the sex of the bearer: in males, it may follow the classical pattern of sexual selection, functioning in mate choice and male-male competition, while in females, plumage colours do not seem to be influenced by male choice, but function in same-sex competitive contexts. Third, it highlights that song has convergent functions in both sexes, as females and males have similar song structure and used song year-round in identical contexts for within-pair communication and joint territorial defence. The fact that females and males sing and have bright colours year-round in parallel with their territorial and breeding behaviour, suggests that individuals use their traits to maintain (sexual and non-sexual) resources.

This work highlights the importance of studying and considering the fundamental differences in females and males, a necessary step for a realistic understanding of ornament expression, and contributes to the ongoing discussion on the evolution of elaborate female signal traits.

DECLARATION

This is to certify that

(i) The thesis comprises only my original work except where indicated in the preface,

(ii) Due acknowledgement has been made in the text to all other material used,

(iii) The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed by

Ana Leitão

Ana M. V. Leitão

February 2019

“I consider it to be the most beautiful species of the genus yet discovered (...)”

Gould, 1852. Proceedings of the Zoological Society of London, 1850, p. 277

PREFACE

This thesis is written as a series of four manuscripts for publication (Chapters 2-5), preceded by a chapter introducing the study and its aims (Chapter 1) and concluding with a general discussion integrating the entire body of work (Chapter 6). This approach led to some repetition, mainly in the methods sections of the manuscripts.

In all chapters, I am the principal investigator and primary contributor to all aspects of the work. However, I could not have completed many aspects of my research without the generous assistance of others. Many volunteers worked on this project as field assistants under my supervision. Fieldwork was remote and the area had several challenges, so most of these tasks could not be carried out alone. In addition, several people taught me how to do the molecular work for Chapter 4, though I conducted the lab work and analysis myself. Everyone who assisted me with the project is listed in the acknowledgments.

Chapters 2 and 3 are published journal articles and are included with the permission of all-co-authors. These are:

Ana V. Leitão, Michelle L. Hall, Brian Venables & Raoul Mulder (2019). Ecology and breeding biology of a tropical bird, the Lovely fairy-wren (*Malurus amabilis*). *Emu*, 119, 1-13. (Chapter 2)

Ana V. Leitão, Michelle L. Hall, Kaspar Delhey, Raoul Mulder (*in press*). Female and male plumage colour signals aggression in a dichromatic tropical songbird. *Animal Behaviour* (Chapter 3)

For Chapter 2, all authors assisted with data collection, and Michelle Hall, and Raoul Mulder contributed to the discussion of results and manuscript preparation. Three anonymous reviewers and editor provided comments to the manuscript. For Chapter 3, Michelle Hall and Raoul Mulder contributed to the experimental design, Kaspar Delhey assisted with the colour analysis techniques, and all authors contributed to the discussion of results and manuscript preparation. Two anonymous reviewers and editor provided comments to the manuscript. The content of the published manuscripts, as presented within this thesis, are unchanged from the published material.

Chapters 4 and 5 are prepared for publication and will be submitted soon. These are:

Ana V. Leitão, Michelle L. Hall & Raoul Mulder (*in preparation*). Pairing, paternity and reproductive success in relation to plumage colouration in Lovely fairy-wrens. (Chapter 4)

Ana V. Leitão, Raoul Mulder & Michelle L. Hall (*in preparation*). Form and function of female and male song in Lovely fairy-wren. (Chapter 5)

For Chapter 5, Michelle Hall assisted with sound and data analysis. In both chapters, all authors contributed to the experimental design, discussion of results and manuscript preparation.

The research reported in this thesis was conducted with approval from the Animal Ethics Committee at the University of Melbourne (register 1613868.1). Fieldwork was carried out under licence from Queensland Parks and Wildlife Service (WISP13237913), and birds were banded under Australian Bird and Bat Banding Scheme banding permits. Funding was provided by the Australian & Pacific Science Foundation (APSF1406 to RM and MLH), Birdlife Australia and Stuart Leslie Bird Research Award (2015 to AVL), and the Australian Research Council (DP150101652 to RM).

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This PhD project was made possible with the support of many people that accompanied me not only on these last 4 years but also all other years that preceded it, people that helped and inspired me in my personal and professional journey. Thank you all!

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Thank you to my advisory committee: Kaspar Delhey for mentoring me in everything related with colour and for supporting me all the way; Mark Elgar for your support and enthusiasm about my project. Thank you also to Kristal Cain, my “mini-boss”, for your wise advices and rich discussions.

I was lucky enough to study with a great model species that is endemic to Cape York Peninsula. That also meant that after a couple of months of arriving in Melbourne and Australia, I had to move to Cairns (a 3000 km epic road trip). Being far was not supposed to be easy, but many people made this journey smooth, made Cairns my second home, and ignited my love for the tropics. My sincere thanks to Brian Venables, who is an essential pillar in all this project, and besides all the valuable local knowledge about the birds and support during fieldwork, him and his lovely family made me feel at home. Also, many thanks to Pippy Cannon for all the bird love, special dinners, and for trusting me with your beautiful house and “Hi-O silver” car during wet season fieldwork; to Patrick de Geest (Eyes on Wildlife), for your friendship, all the beautiful photos of the Lovelies, and for the company on the journey of publishing our paper about cuckoos and Lovelies; to Lucy Friend (Cairns Airport) for your great support and interest in this project; and all local bird people, that helped with find Lovelies, made me company on birding and spotlighting sessions.

The fieldwork was somehow a journey through the unknown, since we didn’t know that much about the Lovelies. Although the beautiful scenery and birds, the days were wet, hot, humid, full of mosquitoes, and mud. Collecting the data in this scenario was only possible with the assistance of a great group of people who travelled from overseas to become field assistants with the Lovely fairy-wren project: Rachel Shepherd (3 months), Gaia Marini (1 month), Phil Chaon (3 months), Irene Mestre (5 months), Zoe Zelazny (2.5 months), Luke Nelson (2.5 months), Kelsey Bell (1 month) - thank you all for contributing immensely to this project, your support, enthusiasm and passion made everything much easier!

In Melbourne a core support was crucial, specially at my final stages of analysing and writing. I would like to thank the Mulder Lab and ex-members: Annie, Farley, Ashton, Kim, Kajanka, Kaya, Izzie, Amy, and specially Timon, for your companionship, fun moments, love for birds and science. I sincerely thank Andrew Katsis and Timon for their patient when teaching me the perks of DNA extraction and sexing. Thanks to Chris Smith for training me in fairy-wrens!

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I was lucky enough to be in the best PhD room in the Bio4 building: thank you to Karla, Henry, Kathryne for your warmth support, and a special thanks to Jed for the friendship and rich discussions. Thanks to all the colleagues in the Bio4 building, for Zoopa and the Friday beers, ShutUp&Write sessions, BioPS pizzas, and corridor conversations. Thanks to all the University of Melbourne demonstrating teams (specially 1st year Biology!) for training me in a very useful set of skills and for your great support. Thanks to GSA, all the thesis writing bootcamps team including Lila, that helped me get here. Thanks to my Portuguese girls Francisca and Bárbara for the beautiful lunches and long conversations in campus. Much love for all the rock-climbing people that I had the privilege to meet these last months and made my mind be free when I needed, “I appreciate you all”! Bisous to Blandine, for your friendship.

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Last but not least, I would like to thank my family, grandpas, my beautiful animals, and specially my Mom, Dad, and Sis, because they are my greatest supporters, they live intensively everything that I do and they love unconditionally what I am. *Obrigada por tudo, amo-vos muito*! Special thanks to my best friend and sis Inês for the epic trips around the world and for always reading my work with great interest, I am very grateful! To my added family Dilva, Osvaldo pai, Diana, João, Telmo, and my niece Maria, for all your love and support and letting me take your son, brother, and uncle from you, *beijinho grande, muito amor*!

Words are not enough to describe the support of my best friend and love Osvaldo, who came to the other side of the world to support my passion, and even if that was not enough, had to be far away from me for months when I was doing fieldwork. Thank you for always encouraging my dreams, for making me smile and feel loved also when things get hard, and for making me believe that everything is possible when I am at your side. I feel like this journey has been one of the greatest times of our lives, and this could only be possible because we have each other. *Obrigada meu amor* *

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Chapter 1

General introduction

General Introduction

"The sexual struggle is of two kinds; in the one it is between the individuals of the same sex, generally the male sex, in order to drive away or kill their rivals, the females remaining passive; whilst in the other, the struggle is likewise between the individuals of the same sex, in order to excite or charm those of the opposite sex, generally the females, which no longer remain passive, but select the more agreeable partners."

Charles Darwin (1871), pp 398

Darwin's (1871) theory of sexual selection inspired numerous studies that provided a successful explanation for how elaborate phenotypic traits evolved in male animals. Males with elaborate traits, such as ornaments or weapons, are often more successful when competing for a partner or preferred as mates, resulting in increased chances of mating success (Andersson, 1994; Darwin, 1871). Although such phenotypic traits are more commonly seen in males, they are also expressed in females across many taxa (Amundsen, 2000a), and are far from unusual even in species with conventional sex roles (Amundsen & Pärn, 2006; Clutton-Brock, 2007; Clutton-Brock, 2009; Heinsohn, Legge, & Endler, 2005; Rosvall, 2011). A traditional focus on male ornamental traits means that comparatively little is known about the role of such traits in females. Understanding how trade-offs and selective pressures shape female ornamentation is crucial to advance our understanding on trait evolution and female and male similarities and differences (Amundsen, 2000a; LeBas, 2006).

Female ornamentation in birds

> *"In various classes of animals a few exceptional cases occur, in which the female instead of the male has acquired well pronounced secondary sexual characters, such as brighter colours, greater size, strength, or pugnacity. (...) Certain female birds have thus been rendered more highly coloured or otherwise ornamented, as well as more powerful and pugnacious than the males, these characters being transmitted to the female offspring alone."*
>
> Charles Darwin (1871), pp 276

Why have studies of female ornaments been uncommon? One of the reasons has been a geographical research bias, commonly focusing on temperate northern hemisphere species (Slater & Mann, 2004) compared to other geographical regions such as the tropics, where female ornamentation seems to be more common (Price, Lanyon, & Omland, 2009; Riebel, 2003; Stutchbury & Morton, 2008). Consequently, documentation of female ornamentation is underrepresented in the literature and its function remains poorly understood. Nevertheless, even in temperate regions, evidence has gradually been accumulating that female birds sing (Krieg & Getty, 2016; Langmore, 1998; Riebel, 2003) and their plumage colours can signal quality and serve a function (Amundsen & Pärn, 2006).

The geographic bias seems to have contributed to a general misconception that a colourful or singing bird is always a male. This is because it is typically presumed that it is unsuitable for females to invest in ornamentation, due to their commonly high energetic investment in reproduction. Females in general have lower variance in reproductive potential compared to males – from gametes to offspring care (Andersson, 1994; although most birds have biparental care: Cockburn, 2006), and this can alter the strength and form of selection (Trivers, 1972). The production and development of signal traits generally implies a constraint on self-development, maintenance or gamete formation, and may also have associated costs related with fighting and predator attraction, which can reduce survival (Fitzpatrick, Berglund, &

Rosenqvist, 1995). Thus, the costs of expressing ornaments may be high and need to be offset by increased fitness. In males it is usually clear that sexually selected competitive traits increase access to mates and result in higher reproductive success. For females this link is not so discernible, but this still does not exclude the possibility of selection on female traits. For example, female reproductive output is also limited by access to reproductive resources such as parental care or nest sites (West-Eberhard, 1983), which can generate selection and favour the expression of traits that improve access to these resources.

Elaborate ornaments in females have been considered by some researchers as non-functional and as a genetic ‘by-product’ of selection on males (Lande, 1980). According to the genetic correlation hypothesis, variation in the level of dimorphism reflects the intensity of sexual selection acting on males. Females may share the genes that express ornaments in males, but if these ornaments impose costs and do not confer any reproductive advantage (e.g. increased fecundity), natural selection may favour reduced expression (crypsis) of ornamental traits (Kokko & Johnstone, 2002). This remains a plausible explanation for why females sometimes express vestiges of male ornaments.

Nevertheless, although recent analyses have confirmed that female ornamentation is to some degree correlated with male ornamentation (Dale, Dey, Delhey, Kempenaers, & Valcu, 2015), female expression of elaborate traits is not easily explained exclusively as a ‘by-product’ of selection on males. Apart from the sex-role reversed species, where ornamented females are common, males are often the limiting sex and females compete for partners (Flanagan, Johnson, Rose, & Jones, 2014; Geberzahn, Goymann, & ten Cate, 2010), female ornaments are also present in species with ‘conventional’ sex roles (Amundsen, 2000a; Odom, Hall, Riebel, Omland, & Langmore, 2014). These can range from no apparent or vestigial ornamentation, through to females expressing similar ornaments to males, to species in which female express traits that are qualitatively or quantitatively different in expression to those on males (Amundsen & Pärn, 2006; Illes & Yunes-Jimenez, 2009; Langmore, 1998). There is accumulating evidence that female ornamentation is influenced by direct selection: female song and conspicuous plumage is likely to be the ancestral state and phylogenetically widespread in songbirds (Friedman, Kiere, & Omland, 2011; Odom et al., 2014; Price, 2009), and evolutionary changes in ornamental traits have been more common in females than in males (Burns, 1998; Dunn, Armenta, & Whittingham, 2015; Irwin, 1994). Dale et al. (2015) also showed that diversity in avian colouration is driven by selection acting differentially between the sexes. Together, these findings raise the question of how these selection patterns arise.

Sexual selection

Theoretical and empirical studies have suggested that female ornamentation can evolve through sexual selection, either through male mate choice (intersexual selection) or female-female competition (intrasexual selection), in similar processes to those functioning in males (reviewed in Amundsen, 2000b; LeBas, 2006).

There is empirical support for selection of traits in mutual mate choice (reviewed in Courtiol, Etienne, Feron, Godelle, & Rousset, 2016; Kraaijeveld, Kraaijeveld-Smit, & Komdeur, 2007), in which both sexes may often discriminate potential mates based on traits. For example, in gouldian finches *Erythrura gouldiae*, females and males discriminated and paired with more elaborate or similar morpho-types (Pryke & Griffith, 2007). In the crested auklet *Aethla cristatella*, both males and females performed more sexual displays to models of the opposite sex with accentuated crests (Jones & Hunter, 1993). In blue tits *Parus caeruleus*, both females and males preferred UV reflecting partners (Hunt, Cuthill, Bennett, & Griffiths, 1999).

Regarding song, to my knowledge, no study has yet tested whether there is mutual mate choice based on song traits.

By selecting high quality mates, females can gain direct benefits such higher survival of the offspring or parental care (Byers, Akresh, & King, 2016; Hill, 1991; Montoya & Torres, 2015), or indirect benefits such as parasite resistance (Doucet & Montgomerie, 2003; Hamilton & Zuk, 1982; Mougeot, Perez-Rodriguez, Martinez-Padilla, Leckie, & Redpath, 2007). Males can also be selective when choosing their mate (Amundsen, Forsgren, & Hansen, 1997; Edward & Chapman, 2011; Griggio, Devigili, Hoi, & Pilastro, 2009), and benefit from female fecundity (Bonduriansky, 2001; Griggio, Valera, Casas, & Pilastro, 2005), or higher reproductive success (Kraaijeveld, Gregurke, Hall, Komdeur, & Mulder, 2004). Mutual mate choice is likely to occur in species that have high variance in individual quality (Kokko & Johnstone, 2002) and in species with socially monogamous mating system and bi-parental care, in order to increase reproductive success (Amundsen & Pärn, 2006; Kraaijeveld et al., 2007).

Female ornaments can also function as signals in same-sex competitive contexts over mates, when availability of partners is a limitation or there is variation in quality (Clutton-Brock, 2007). In birds there have been fewer studies in the context of female competition for mates, with more examples in sex role-reversed species. For example, in the polyandrous Eurasian dotterel *Charadrius morinellus*, brighter females compared to duller females win more fights, court more males, and pair earlier in the season with brighter males (Owens, Burke, & Thompson, 1994). In conventional sex-roles, the relation between female-female competition and reproductive resources is not easy to disentangle, though several examples can be found for female-female competition in general, more likely to be related with reproductive or ecological resources (see below). Ultimately, both same-sex contests and mate choice are forms of competition for reproductive resources.

Social selection

It is likely that sexual preferences for phenotypic traits could be extended to nonsexual social preferences (Cardoso et al., 2014; Tóth & Griggio, 2011), as competition can extend to other social functions (Kraaijeveld et al., 2004; Leitão, Ferreira, Funghi, Trigo, & Mota, 2015). Therefore, trait evolution may be better comprehended through the concept of social selection (Amundsen, 2000b; LeBas, 2006), which encompasses selection due to sexual and nonsexual interactions, and suggests that ornamentation may have evolved as a signal of competitive ability for both mates and other non-sexual but essential resources such as territories or food (West-Eberhard, 1979, 1983). Instead of considering solely how female ornaments can be included within a sexual selection definition, social selection broadens the concept and can provide a different perspective into the evolution and adaptive significance of female ornamentation. In this scenario, empirical studies have shown that female elaborate traits are used during competition for mates or other resources (Cooney & Cockburn, 1995; Crowley & Magrath, 2004; Kraaijeveld et al., 2004; Murphy, Rosenthal, Montgomerie, & Tarvin, 2009b; Swaddle & Witter, 1995; Tobias, Gamarra-Toledo, García-Olaechea, Pulgarín, & Seddon, 2011). For example, studies have linked female ornamentation with aggressiveness towards simulated female intruders, like increased song rate in response to female playbacks (Cain & Langmore, 2015; Illes & Yunes-Jimenez, 2009; Krieg & Getty, 2018; Mulder et al., 2003), or increased aggression towards colour-manipulated female models (Enbody, Boersma, Schwabl, & Karubian, 2018; Midamegbe, Grégoire, Perret, & Doutrelant, 2011; Morales et al., 2014; Murphy, Hernández-Muciño, Osorio-Beristain, Montgomerie, & Omland, 2009a).

Signal content

Variation in ornament expression may provide a signal in social or sexual contexts, if these traits convey reliable information on some aspect of quality and fitness (Amundsen, 2000a; Cardoso, 2013; Hamilton & Zuk, 1982; Zahavi, 1975). Where there are conflicts of interest between signallers and receivers, honesty in signals (i.e. when the signal accurately represents the quality of the signaller; reviewed in Searcy & Nowicki, 2005; Vehrencamp, 2000), can be maintained via production costs, maintenance costs, higher vulnerability to predators (handicap signals; Zahavi, 1975, 1977), or costs imposed by receivers through social punishment (Hurd & Enquist, 2005; Tibbetts, 2013; Tibbetts & Dale, 2004).

Several studies in different taxa have demonstrated that female ornamentation can have a signalling function (Baldauf, Bakker, Kullmann, & Thünken, 2011; Domb & Pagel, 2001; Gladbach, 2010; Stuart-Fox & Goode, 2014; Weiss, Kennedy, & Bernhard, 2009). Song rate is positively related with breeding success in the New Zealand bellbird *Anthornis melanura* (Brunton, Roper, & Harmer, 2016), and in the superb fairy-wren *Malurus cyaneus* (Cain, Cockburn, & Langmore, 2015). Regarding colour traits, in the common eider *Somateria mollissima*, female white plumage is correlated with the tolerance of reproductive costs (Hanssen, Folstad, & Erikstad, 2006); in the northern cardinal *Cardinalis*, wing brightness reflects parental quality (nestling provisioning) (Linville, Breitwisch, & Schilling, 1998). In the great tit (*Parus major*), more ornamented females produce higher quality offspring (Remeš & Matysioková, 2013), and in the tree swallow *Tachucineta bicolor* female plumage colour reflects their resilience to stress (Taff, Zimmer, & Vitousek, 2019). Female benefits may be offset by constraints, and a comparative study revealed that female plumage brightness is associated with higher nest predation (Martin & Badyaev, 1996). Similarly, Kleindorfer et al. (2016) found that in the superb fairy-wren, female song rate predicted egg and nestling predation, evidencing high fitness costs for song rate. Collectively, these studies indicate that ornamentation can signal quality and be associated with reproductive benefits but also costs such as predation.

Malurus genus

The *Malurus* genus includes 12 species (Joseph, Edwards, & McLean, 2013; Marki et al., 2017; McLean, Toon, Schmidt, Hughes, & Joseph, 2017) (Figure 1), distributed throughout Australia and Papua New Guinea, occupying variable types of habitats (Rowley & Russell, 1997).

The fairy-wrens are remarkable for their social organisation: all species in this group are territorial group-living and facultative cooperative breeders. Groups often contain multiple non-breeding individuals (also referred as subordinates or helpers) which are usually male but sometimes female, and assist the dominant pair in their reproductive efforts (Rowley & Russell, 1997). These species are also renowned for their remarkable mating systems, which include perhaps the most extreme variation in frequency of extra-pair paternity found within one genus of birds: at one extreme, 76% of superb fairy-wren offspring are sired by a male outside the social pair bond (Mulder, Dunn, Cockburn, Lazenby-Cohen, & Howell, 1994), while at the other extreme, only 4.4 % of offspring resulted from extra-pair mattings in the purple-crowned fairy-wren *Malurus coronatus* (Kingma, Hall, Segelbacher, & Peters, 2009).

All fairy-wren species are sexually dichromatic, with females and males exhibiting high variation in plumage colours across species and between sexes. All males and some females have showy colours (Enbody, Lantz, & Karubian, 2017; Rowley & Russell, 1997). Most males display seasonal plumages (Peters, Kingma, & Delhey, 2013; Rowley & Russell, 1997), and females appear to choose males that moult earliest as extra-pair mates (Brouwer, van de Pol, Atema, & Cockburn, 2011; Cockburn, Osmond, & Double, 2008; Mulder & Magrath, 1994). Johnson et al. (2013) showed that female colouration in fairy-wrens and other *Maluridae* has changed drastically in some lineages and little in others, generating patterns of dichromatism, which suggests that males and females are under different selective pressures. Differences in plumage colour have been proposed to be under sexual selection in males (Friedman & Remeš, 2015) and under natural selection in females (Medina et al., 2017).

The suggested differences in selective pressures may also affect song in fairy-wrens. Songbirds are thought to have originated in Australasia (Barker, Cibois, Schikler, Feinstein, & Cracraft, 2004), where female song is common (Robinson, 1949). All males and females fairy-wren sing (Rowley & Russell, 1997). Fairy-wrens are basal oscines, part of the earliest branches of the songbird phylogeny (Barker et al., 2004; Jetz, Thomas, Hartmann, & Mooers, 2012), which makes them an ideal model species for studies on song, though most studies have been conducted in species of higher level of phylogenetic classification such as zebra finches *Taeniopygia guttata* or song sparrows *Melospiza melodia.*

Within the *Malurus*, species differ in their acoustic structure and show a high degree of divergence in their song traits, including song complexity (Greig, Price, & Pruett-Jones, 2013). Song has been increasingly studied in males (Dowling & Webster, 2018; Greig & Pruett-Jones, 2010; Hall, Kingma, & Peters, 2013) and females (Cain & Langmore, 2015; Colombelli-Négrel, 2016; Cooney & Cockburn, 1995; Greig & Pruett-Jones, 2008; Kleindorfer et al., 2016). Still, it remains poorly known how much variation exists between sexes in their structure, complexity and contexts in which song occurs, particularly because few studies have been conducted on both sexes simultaneously (Dowling & Webster, 2015; Hall & Peters, 2008; Kleindorfer et al., 2013).

Fairy-wrens have become a model system for topics such as sexual selection, mating systems, and male ornamentation (Baldassarre, Thomassen, Karubian, & Webster, 2013; Johnson & Pruett-Jones, 2018; Karubian, 2002; Kingma et al., 2009; Mulder et al., 1994; Tarvin, Webster, Tuttle, & Pruett-Jones, 2005). Like in many other species, the main focus has been on males, with some exceptions (Colombelli-Négrel, 2016; Cooney & Cockburn, 1995; Double & Cockburn, 2000; Enbody et al., 2018; Hall & Peters, 2008; Karubian, 2013; Kleindorfer et al., 2013; Peters, 2007). The variability of structure and functions of song and plumage colour in the genus, together with their complex social interactions and wide range of different geographical distributions and habitats, provides a great opportunity to advance our understanding on signal evolution in females and males.

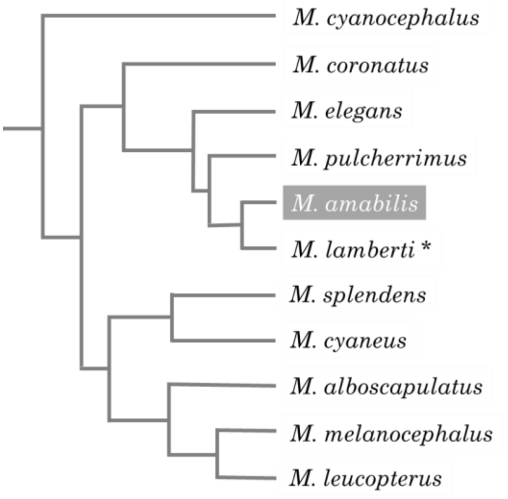

Figure 1. Ancestral state for 11 Malurus species, based on the most recent phylogeny (Marki et al., 2017). * Recent genetic analysis outlines the separation of purple-backed fairy-wren M. assimilis from variegated fairy-wren M. lamberti (McLean et al., 2017), resulting in 12 Malurus species.

The Lovely fairy-wren as a model

The lovely fairy-wren, *Malurus amabilis,* was first described by John Gould (1850). The bright plumage of female lovely fairy-wrens led John Gould (pp. iv, 1869) to initially describe male and female incorrectly as two different species (Figure 2):

> *“Mr James Cockerell (...) believes that my Malurus amabilis and M. hypoleucus are male and female of the same species, for he has seen and shot them in company many times – the M. amabilis being the male, and M. hypoleucus the female. If this should prove to be the case, it will be contrary to what I have hitherto believed to be an invariable law with these birds; for I have always supposed the females of the variegated Maluri, like the Common Superb Warbler (Malurus cyaneus), to be of a nearly uniform brown, (...)*”

This was then confirmed to be true, and the species were merged.

Before my research, little was known about the ecology and behaviour of the lovely fairy-wren (Rowley & Russell, 1997); most of the knowledge was based on casual observations, some made over a century ago (Alexander, 1926; Barnard, 1911; Macgillivray, 1914; White, 1946).

The lovely fairy-wren is endemic to the Cape York Peninsula, in Far North Queensland, Australia, occupying mainly coastal areas (Rowley & Russell, 1997). The species is socially monogamous, but like other fairy-wrens it is possible that exhibits extra-pair mating, although rates of extra-pair fertilisations were unknown before this thesis research.

Female lovely fairy-wrens express the most colourful plumage of all the female Australian fairy-wrens. A recent comparative analysis has shown that conspicuous female plumage in this species experienced dramatic evolutionary change resulting in reduced dichromatism, suggesting evolutionary selection for female plumage ornamentation (Johnson et al., 2013). However, what mechanisms drove these changes remains unknown. Also, female song seems to be common in this species (Rowley & Russell, 1997), but female and male song has never

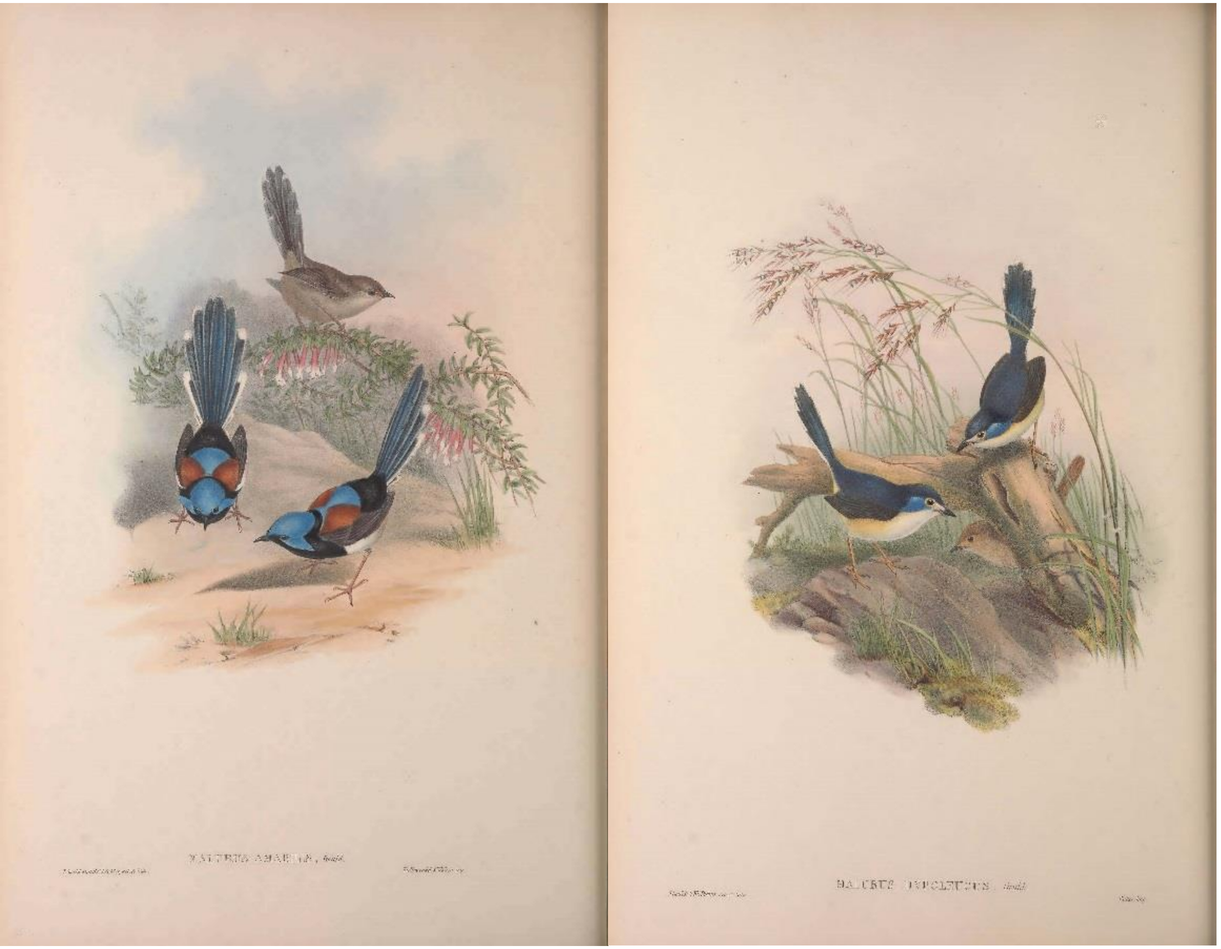

Figure 2. John Gould’s plates. On the left lovely fairy-wren *M. amabilis* (male) and on the right the assumed distinct species fawn-breasted superb warbler *M. hypoleucus* (lovely fairy-wren female), which were then later merged as one species. Illustrations from The Birds of Australia (Gould, 1869), obtained from the Biodiversity Heritage Library, contributed by Smithsonian Libraries. Copyright status: public domain. www.biodiversitylibrary.org

been investigated, with the sole exception of a comparative study on acoustic structure of male fairy-wrens (Greig et al., 2013)

Overall, the lovely fairy-wren is ideally suited to investigate not only the functional aspects of song and colouration in this species, but also to provide a significant contribution to our understanding of the processes that drive the expression of female and male ornamentation in fairy-wrens, and contribute more broadly to a general understanding of the evolution of female ornamentation.

Research objectives and thesis outline

More studies are required to clearly understand patterns that guide us to general understanding of female ornament function and evolution. In particular, studies in which both sexes are conspicuously ornamented are scarce (Amundsen & Pärn, 2006), and studies rarely have been conducted in both male and female in a single system. It is likely that the explanation proposed for elaborate male ornamentation may not apply to females, due to important differences between sexes in selective pressures and investment trade-offs in ornamentation. Studying traits using similar procedures in females and males may help understand sex-differences in traits, possible mechanisms driving these differences, and functional and evolutionary consequences.

Additionally, systematic studies are needed to investigate hypotheses of signalling function in different ornamental traits, such as colourful plumage and song. Song is detectable at longer distances in comparison to colouration, which is a more close-range signal. Consequently, the evaluation of these two different traits implies distinct perceptions and ecological scenarios.

In this thesis I investigate the form and function of female and male plumage colour and song. My thesis research employed field observations, behavioural experiments, and genetic analysis, to test the adaptive function and mechanisms for the evolution of female and male ornamentation in lovely fairy-wrens. I explicitly contrast females and males so that we can address, in the light of the abundant work done on males, how females may, or may not differ from males.

To understand the mechanisms related with ornamental traits, we first must understand the basic ecology, breeding biology and behaviour of the species. Although many other members of the *Malurus* genus have been the subject of extensive research (Buchanan & Cockburn, 2013; Rowley & Russell, 1997), a detailed description of the ecology of the Lovely fairy-wren prior to this work was lacking. In **Chapter 2**, I provide a comprehensive overview of the ecology, behaviour and breeding biology of the Lovely fairy-wren and compare these traits to those of the other *Malurus* species. The detailed information on the species natural history provided in this chapter is essential for interpretation of later chapters.

In **Chapter 3**, I describe plumage colouration in females and males and experimentally investigate whether plumage colouration in females and males is a signal, functions in a competitive context, and whether there are sex differences in signal function. I test this through experiments, where I created a novel competitive environment using a mirror image stimulation test. I first measured the colour reflection and applied psychophysical models of avian vision to describe colour in both sexes. I then tested the degree of aggression birds expressed when exposed to mirror image stimulation and if it was related to natural plumage colour. Finally, I manipulated individual plumage colour and examined whether males and females behaved differently in response to their mirror image reflection.

The role of plumage colour in competition is not necessarily exclusive, and plumage can play a role in other contexts such as inter-sexual interactions. To understand if mate-choice is a selective driver of plumage colouration in this species, in **Chapter 4** I first examine if plumage signals individual quality and incurs survival costs. I then investigate whether males and females paired assortatively based on plumage colour and whether this resulted in increased reproductive and paternity success. To objectively measure reproductive success and paternity, I used molecular tools to describe the genetic mating system of this species.

Studying two signal traits in distinct signalling scenarios provides an opportunity to better understand the potential pressures operating in favour of ornamental traits in females. In **Chapter 5** I step away from plumage colouration and look at the function of song in females and males. I used observational data to describe their song structure and examine sex-specific variation in song rate across breeding and non-breeding stages and seasonal variation. I also used experimental data to examine female and male response to simulated territorial intrusion with a playback experiment simulating solo intruders of either sex, singing songs that varied in complexity.

I conclude the thesis in **Chapter 6**, by providing a brief synthesis of the main findings of earlier chapters and outlining ongoing projects and suggestions for future research.

Chapter 2

Ecology and breeding biology of a tropical bird, the Lovely fairy-wren (*Malurus amabilis*)

Ana V. Leitão, Michelle L. Hall, Brian Venables, Raoul Mulder

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Abstract

The Lovely Fairy-Wren (*Malurus amabilis*) is endemic to the wet tropics of Australia and is one of 11 species in the genus Malurus. Despite the large number of studies on fairy-wrens, little is known about the Lovely Fairy-Wren. This study provides the first detailed description of its ecology, behaviour, and breeding biology. Lovely Fairy-Wrens displayed breeding behaviour characteristic of tropical birds, with groups maintaining territories and breeding year-round, small clutch size (two to three eggs), long juvenile dependence (2 months) and high adult breeder survival (86%). They breed cooperatively, and groups formed when male (but not female) offspring delayed dispersal and remained in their natal group as subordinates. Groups were typically small (2.5 ± 0.8 individuals), possibly because productivity was low: 29% of the monitored groups produced at least one fledgling per year. Males provided high levels of parental care and this, together with low extra-pair courtship and petal displays, suggests that this species may not be as promiscuous as other fairy-wrens. Unlike other Australian fairywrens, males maintained their brightly coloured adult plumage year-round after initial acquisition. This lack of seasonal moult into dull plumage, coupled with the unusually colourful plumage of females in this species, suggests that the impact of natural selection on the plumage colour of both sexes may be lower in this species than in their congeners. We discuss similarities and differences in life-history and morphological traits between the Lovely Fairy-Wren and other Malurus species.

Keywords: Australian birds; behavioural ecology; breeding; bright plumage; Fairy-wren; Malurus; moult; tropical birds

Introduction

The genus *Malurus* (fairy-wrens) occurs throughout Australia and New Guinea, and has become a globally recognised model for studies of evolutionary ecology, including cooperative behaviour, mating systems, male ornamentation, and brood parasitism (Buchanan & Cockburn, 2013). However, several species and subspecies of the genus have never been studied, and knowledge of species of the Northern tropics of Australia and of New Guinea is particularly poor. These under-studied Malurus may challenge the generality of conclusions drawn from their better-studied temperate counterparts. Some characteristics that already differ conspicuously in these unstudied species include the colourful plumage of female Lovely Fairy-Wrens and the variegated subspecies *rogersi* and *dulcis*. Additionally, tropical species may exhibit life-history traits that differ from temperate climate species and represent potentially important test cases: the tropics are generally characterised by lower seasonality in the availability of food and high rates of nest predation, which have been related to 'slow' life-history traits (Martin, 1996), like higher adult survival rates (Johnston, Peach, Gregory, & White, 1997) and extended parental investment (Russell, Yom-Tov, & Geffen, 2004).

The Lovely Fairy-Wren (LFW) belongs to the 'chestnut-shouldered group', one of the three clades in the genus (the other two are the 'bi-coloured' and the 'blue group'; Table 1), and is the north-eastern form of that group, occupying rainforest margins in the tropical North Queensland (Rowley & Russell, 1997). Females and males are highly colourful and sexually dichromatic (Figure 1(a, b)), while juveniles display a duller version of the female plumage (Figure 1(c); (Higgins, Peter, & Steele, 2001)). Besides differences in habitat and physical characteristics compared to other fairy-wrens, little is known about LFW ecology and behaviour (Rowley & Russell, 1997). Here, we describe the ecology, behaviour, and breeding biology of the LFW based on studies of colour-banded populations over 5 years.

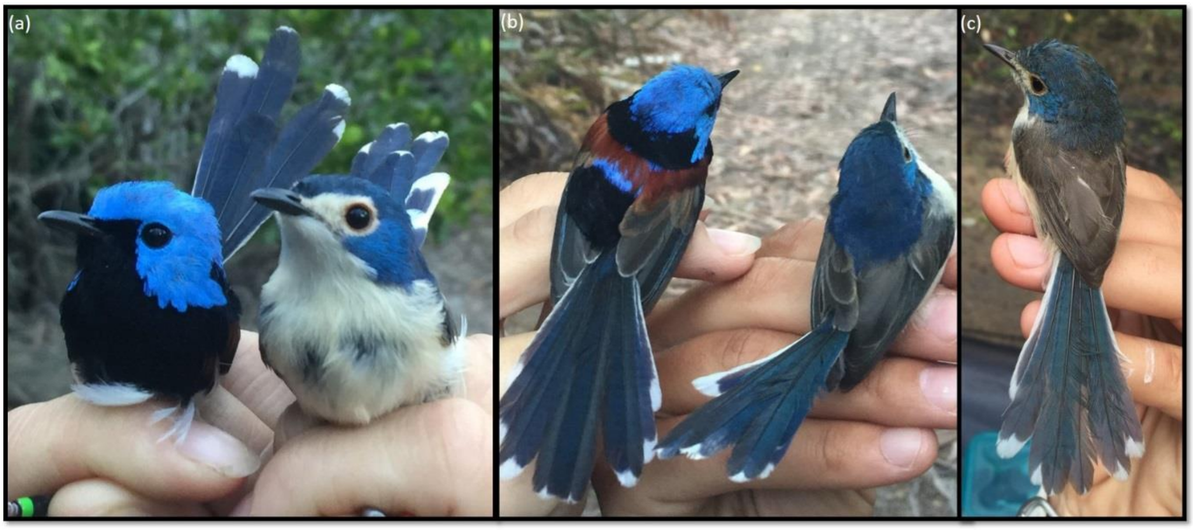

Figure 1. (a) Male and female Lovely fairy-wren Malurus amabilis colour differences, frontal view and (b) dorsal view. (c) Dependent juvenile with duller version of the female plumage.

Table 1. Overview of different attribute of life-history, ecology, and breeding biology in Fairy-wrens (FW). A dash (–) indicates no published data available. ‘Territory’, ‘density’, and ‘group size’ show average and/or range. References: 1. Del Hoyo, Elliot et al. (2007); 2. Rowley and Russell (1997); 3. Schodde (1982); 4. Higgins, Peter et al. (2001); 5. Tidemann (1990); 6. Johnson (2016); 7. Rowley and Russel (2002); 8. Rowley and Russell (1988); 9. Rowley and Russell (1993); 10. Kingma, Hall et al. (2009); 11. Van Doorn (2007); 12. Peters, Kingma et al. (2013); 13. Hidalgo, Hall et al. (2016); 14. Russell and Rowley (1993); 15. Brooker and Rowley (1995); 16. Mulder and Magrath (1994); 17. Varian-Ramos, Lindsay et al. (2012); 18. Lantz, Boersma et al. (2017); 19. Karubian et al. Karubian, Sillett, and Webster (2008); 20. Rathburn and Montgomerie (2003); 21. Rowley and Russell (1995).

	Territory size (ha)	**Density (birds/ha)**	**Divorce (%)**	**Survival (%)**	**Group size**	**Helper sex**	**Pre-breed moult duration**	**Seasonal plumage**	**Clutch size**	**Incubation (days)**	**Fledge (days)**	**Depend. (months)**	**References**
Chestnut-shouldered group													
Lovely Fairy-wren *Malurus amabilis*	1.7, 0.3-4.6	1, 0.6-1.3	10.9	86	2.5; 2-5	♂	2-11 months	No	2-3	12-16	12-14	2	This study
Variegated FW M. lamberti	3.7; 3.4-4.4	0.1-2.1	-	78	2-10	♂ (♀ unkn.)	2-4 weeks	Yes	2-4	14-16	10-12	1	[1] [2] [3] [4] [5] [6]
Blue-breasted FW *M. pulcherrimus*	2.5; 1.5-4	0.5-2.2	4.7	53-60	2.4; 2-4	♂♀	3-4 weeks	Yes	2-4	14-15	10-12	1	[1] [2] [3] [4] [5] [7]
Red-winged FW *M. elegans*	1.2; 0.4-2.4	3.2; 2.8-3.6	-	78-79	4; 2-9	♂♀	<1 month	Yes	1-3	14-15	11-12	1	[1] [2] [4] [8]
Blue group													
Purple-crowned FW *M. coronatus*	0.4; 156-296m length	18; 14-28 birds p/ km	8	70-81	3.3; 2-11	♂♀	46 days	Yes	1-4	14	10-13	2	[1] [2] [9] [10] [11] [12] [13]
Emperor FW *M. cyanocephalus*	-	-	-	-	2-5	♂ (♀ unkn.)	-	No	1-4	-	-	-	[1] [2] [3]
Splendid FW *M. splendens*	4.4, 1-8.7	0.4-1.1	3.4	59-70	3.3; 2-8	♂♀	-	Yes	2-4	12-17	10-13	1	[1] [2] [14] [15]
Superb FW *M. cyaneus*	1.6; 0.5-2.3	0.16-9	4	45-69	2.9; 2-9	♂ (♀ rare)	25 days	Yes	2-4	14	10-14	1	[1] [2] [4] [5] [16]
Bi-coloured group													
White-shouldered FW *M. alboscapulatus*	0.1-2	-	-	-	2-8	♂ (♀ unkn.)	-	No	2-4	11-12	12-13	1	[1] [2] [3]
Red-backed FW *M. melanocephalus*	<1	0.13-2.72	-	56-62	2-4	♂ (rare)	25 days	Yes	2-5	12-13	11-12	1	[1] [2] [3] [4] [17] [18] [19]
White-winged FW *M. leucopterus*	3.3; 1.6-6	0.8	-	45-64	2-6	♂	-	Yes	2-4	13-14	10-11	1	[1] [2] [4] [5] [20] [21]

Methods

Study species

The LFW is endemic to the Cape York Peninsula in Australia, occupying dense coastal vegetation such as rainforest edges, paperbark swamps, shrubby woodlands, and mangroves (Higgins et al., 2001).

Like other fairy-wrens, the LFW is a cooperative breeder (White, 1946), characterised by the presence of breeding and non-breeding mature individuals that contribute to the reproductive effort of the group (the latter commonly referred as ‘subordinates’ or ‘helpers’). There are reports of nests or fledglings found from July to April (Macgillivray, 1914; White, 1946), possibly with breeding peak in the dry season (Schodde, 1982). The average life span of the LFW is not known, but maximum life span recorded is 12 years (Australian Bird and Bat Banding Scheme online database).

Study area and population

Research was conducted from 2013 to 2017, in the Cairns region, Far North Queensland, Australia (16.87°S 145.75°E). The region is characterised by a tropical monsoonal climate, with two distinctive seasons: dry winters (May - October) and wet summers (November - April), with 86% of 1992 mm average annual rainfall falling in the wet season. Temperatures vary from an average daily minimum of 24°C in July to an average daily maximum of 32°C in January, and monthly rainfall averages range between 27–92 mm in the dry season and 93-448 mm in the wet season (Cairns station #031011, Australian Bureau of Meteorology).

We monitored LFW at five main sites (Table 2; Supplementary Figure S1): Redden Island, Airport Reserve, Holloways Beach, Yorkeys Knob Reserve and Smithfield. The study area comprised a total area of 162 ha of complex mosaic of different types of habitat (Table 2), separated by natural (water bodies) and anthropogenic barriers (roads, houses, sugar cane fields). Monitoring commenced at Redden Island in 2013, and at the remaining sites in 2015, except for Smithfield (2016 start). Fieldwork was conducted during two-week field trips in 2013 and 2014 (October-November), to begin establishing a study population, progressing to more intensive monitoring in 2015 (May-December), 2016 (March, July-November) and 2017 (March, August-September).

Table 2. Social composition of territorial groups at each field site: area studied in hectares (ha), number of groups (and birds), mean territory size (and range), neighbouring territories (and range), bird density (n/ha), mean group size (and range), percentage of pairs and sex ratio. Data for 2016 and 2017 field season.

Site	Area studied (ha)	Groups (Birds)	Territory size (range, ha)	Neighbouring territories (range)	Bird density	Group size (range)	% pairs	Sex ratio
Redden island Forest, Mangrove, Woodland, Riparian	29	9 (21)	2.1 (1.1-3)	3.1 (2-5)	0.72	2.3 (2-4)	77.7	1.3
Airport reserve Mangrove, Beach	42	18 (47)	1.2 (0.8-1.6)	2.0 (0-3)	1.12	2.6 (1-4)	50	1.3
Yorkeys Knob Forest, Mangrove, Beach, Sugar cane fields	20	10 (25)	2.0 (1.5-2.5)	1.4 (1-2)	1.25	2.5 (2-5)	70	1.2
Holloways beach Forest, Mangrove, Sugar cane fields	45	13 (27)	2.7 (2.5-4.6)	1.3 (1-2)	0.59	2.3 (1-3)	53.8	1.3
Smithfield Forest, Rainforest edge	26	14 (35)	0.59 (0.3-1.3)	2.9 (2-4)	1.34	2.5 (2-5)	64	1.3
Total / Average	~162	64 (155)	1.7	2.3	1	2.5	63	1.3

Bird capture and measurements

Unbanded adult and juvenile birds were captured opportunistically in mistnets (208 individuals), usually by luring birds with playback of conspecific vocalisations; nestlings were captured in the nest. Birds were individually marked with a numbered metal band (Australian Bird and Bat Banding Scheme) and a unique colour combination to allow individual identification in the wild. Standard morphometric measurements were taken including head and bill length and tarsus length (vernier caliper; ± 0.05 mm), flattened wing chord and tail length (ruler; ± 0.1 mm), and body mass (spring balance; ± 0.25 g). We also checked for presence of body moult (pin feathers), brood patch, and cloacal protuberance. Sex of the bird was recorded based on plumage. We collected blood samples (max. 50 µL) in heparinised capillary tubes from the brachial vein and stored them in 100% ethanol. Sexes were confirmed by DNA analysis (see Supplementary material).

Monitoring and behavioural observations

Colour-banded birds were censused and monitored at approximately weekly intervals during field seasons. For each individual we recorded the ID, group membership, moult, GPS coordinates, role (dominant, subordinate, juvenile), and breeding activity. From 2015, we monitored between 30 and 64 groups (numbers varied due to the addition of sites and/or disappearance of some groups).

Throughout the 2015-2017 field seasons, we carried out focal behavioural observations on each group every week/fortnight (>700 observations of 20 minutes to one hour, usually 0600-1200 hours, totalling >400 h on 46 groups). After recording information from each individual (see above), we followed groups at distance (>5 metres) and noted behaviours and coordinates of where these occurred. Behaviours included feeding, courtship and petal displays, nesting activity, and aggressive behaviours (see Supplementary material and Table S1).

We estimated annual adult survival by recovery rate of banded adult breeders. Probability of detection was high, because we censused the study sites frequently. Emigration rates by breeders were low and any error due to undetected emigrants would mean actual survival rates were higher than our (already high, see Results) estimates.

Dispersal events were recorded when an individual was seen on two consecutive occasions in a different location from its original territory. Divorce was recorded when both original partners were confirmed to be alive, but at least one of them was paired to a different bird.

Habitat, territory size and population density

Territory sizes were determined and mapped by recording coordinates of locations where banded birds were seen and where territorial disputes occurred (>4800 recorded coordinates). Size of study areas and territories were estimated using Google Earth (Google Inc), and Earth Point (http://www.earthpoint.us/). Bird density was calculated for each field site as the total number of birds divided by the total study area, based on the 2016 and 2017 field season.

Nesting behaviour

Over three seasons (2015-2017), we monitored 144 breeding attempts by 15 to 35 groups. Nests were located by following females collecting material and incubating, or individuals carrying food. We measured nest height from the ground, and height width and depth of the nest (tape measure; ± 0.5 cm) to calculate volume (n = 87). We also qualitatively recorded nest visibility 1-2 metres from the side and top (open, partially concealed or concealed) and substrate (described in the results).

Observations at different stages during nesting were used to estimate key nesting parameters (first egg date, hatch date, etc.) based on nests checked every day to three days, to accurately calculate: duration of construction, laying period, incubation, and nestling period (see Supplementary material). We noted the outcome for each nest found: abandoned or destroyed, parasitised, predated and at which stage, and fledgling survival. We also described nestling development and fledgling age and behaviour. Annual reproductive success was quantified as the total number of fledglings that survived for at least a month.

To record predation events, in 2016, we placed motion-activated camera traps (Bushnell Trophy Cam HD Aggressor) 2–4 metres from the front or side of the nest entrance (*n* = 24). Cameras were removed after fledging or predation. We did not observe any cases of apparent abandonment related to the camera trap.

Nest provisioning

Food provisioning and brooding data were obtained in 2015 and 2016 by video recording active nests when nestlings were 3-4 days old (*n* = 17 early stage) and 5-9 days old (*n* = 19 late stage). We placed a small video camera (GoPro HD Hero2) in front of each nest and recorded for one hour in the morning (0700-1230). Adults resumed feeding 30 s to 10 min after camera placement. Individuals could be distinguished on camera by their colour bands. From the recordings, we estimated brooding time and provisioning rates (feedings/hour) of female and male breeders and subordinates.

Statistical Analysis

We compared morphometric parameters of females and males using Mann-Whitney tests, since measurements were not normally distributed.

We used a General Linear Mixed model to test nest provisioning rate for each individual and fitted in the model as explanatory variables nestlings' age (days since hatch), brood size, sex and the interaction between sex and age of the provisioning individual, and presence of subordinate. None of the observed nests had more than one subordinate present. We also included an interaction between age of the nestlings and sex of the provisioning individual because parents may respond differently to nestlings age. Nest attempt, territory and individual identity were included as random factors to account for non-independence of the data. We found no difference between years (ANOVA: $P > 0.3$), and so we combined them. We report the mean ± SD for descriptive statistics throughout.

Results

Population: Habitat, territories, and density

Population density varied between sites (Table 2), averaging 1 bird/ha. Groups were found in varied habitats, from rainforest edges to woodland, paperbark forest, riverbanks, sand-dunes, and mangroves (Supplementary Figure S2). Territories ranged from 0.3 to 4.6 ha (1.6 ± 1.05 ha across all field sites), and on average each territory had 2.3 ± 1.09 neighbouring territories (range: 0-5, Table 2). Most territories contained two or more types of habitat, differing in vegetation density (e.g. mangrove and woodland).

Banded birds were consistently found in the same territories throughout the year and groups remained as cohesive units; birds were never seen forming larger groups or aggregating with other conspecifics during non-breeding periods. Males, females, and subordinates were often observed defending their territory against conspecific intruders (>60 instances), by performing aggressive displays that occasionally culminated in physical aggression, typically directed to same-sex opponents. These interactions were observed in both wet and dry seasons.

On average, 88% territories were maintained from one year to the next and did not vary greatly in size between years. Nine percent of territories (1 of 11) were abandoned in the first year, 0% of 12 in the second, 18% (7 of 38) in the third year, and 12% (4 of 32) in the fourth. Territory abandonment was triggered by the death and/or dispersal of occupants. Neighbouring groups usually, but not always, annexed newly-vacant areas to expand their own territories.

Social organisation and survival: pair bonds, divorce, cooperative breeding, and natal dispersal

The annual survival of adult breeders varied between years (Supplementary Table S2) but was generally high for both sexes: on average 86% survived one year to the next (84.4% of females and 87.5% of males).

Paired adults maintained long-term bonds: on average 62% of monitored pairs continued their partnership from one year to the next (2014: 67%, $n = 6$; 2015: 73%, $n = 11$; 2016: 60%, $n = 30$; 2017: 50%, $n = 22$; pairs with both members banded). Twenty percent of pairs changed breeding partner after one year together, 50% of the pairs were stable for two years, 40% of the pairs were together after three years or more and the average duration of a pair-bond was 2.1 ± 0.9 years (limited by the short duration of the study). Partnerships ended due to disappearance/death of partner (*n* = 21) or divorce (*n* = 7), and following such events, individuals either stayed in their territory (two-thirds) or dispersed (one-third). When breeders dispersed, females moved 94-2725m from their previous territory (508.5 ± 689.84, *n* = 11) and males moved 187-1270m (408.8 ± 431.75, *n* = 4). In all cases of breeder dispersal, females and males occupied a breeder position in their new territory.

The annual divorce rate averaged 10.9% (7 events between 2014-2017). The female left the territory and the male remained in all, except one case where the male left the female.

The average size of groups was 2.5 ± 0.8 (Table 2), and 34.7% of 49 groups included subordinates. To our knowledge, all subordinates were progeny that delayed dispersal and assisted with subsequent reproductive attempts (based on 13 banded dependent young and behavioural observations of groups). Eighty-six percent of 21 subordinate males stayed in the group for more than one year, and at least 61% of the males stayed more than two years; 28% of subordinate males inherited the breeder position in their natal territory. Male natal dispersal occurred typically in complete adult plumage. In all cases, males obtained breeding positions and were found 100-400m close to their natal territory (255.6 ± 98.09, *n* = 6). No subordinate females stayed in their group for more than six months or were seen in their natal group during nesting (five molecularly sexed). Two females were found 2430 and 5340 m away from their natal territories occupying a breeding position, and one other female juvenile was observed >260 metres away in neighbouring territories (after nesting commenced on her natal territory) before disappearing. The remaining two subordinate females in the study disappeared, suggesting they either died or dispersed long distances. Male, but not female subordinates, were seen feeding nestlings and fledglings.

Female and male traits: morphology and plumage

Juveniles of both sexes have a duller version of adult female plumage (Figure 1 c) and a pale brown bill base. Males of known age (*n* = 4) attained full adult bright plumage in their first year, at between 222 ± 5 and 335 ± 13 days of age. The time that males took to fully moult from juvenile to adult male plumage ranged from 2 to 11 months (*n* = 10, average 197.3 days: 55–340 ± 14 days; Supplementary Figure S3 for patterns of male moult), and so moult occurred in most months of the year (Figure 2). Banded adult males maintained bright plumage year-round after initial acquisition, never moulting back to a dull plumage (>400 re-sightings from 2013 – 2017, including 132 in wet season/April, and >150 in dry season/July). This included subordinate males (*n* = 11).

Timing of post-juvenile moult in females is unknown due to subtle differences between juvenile and female adult plumage, that are best distinguished in the hand. Observations of captured subordinate females before dispersing (*n* = 2), showed no sign of juvenile plumage, and all natal female dispersers recaptured had full bright plumage (*n* = 3; Supplementary Figure S4 for an example), suggesting that females disperse after attaining their bright adult plumage. Adult females maintained bright plumage year-round (23 recaptures and >300 re-sightings).

Replacement (moulting) of body feathers was observed in bright adult males and females in all months that birds were captured (May – November, Supplementary Figure S5), though overall female and male appearance did not change throughout the year after attaining their bright adult plumage.

Adult males were significantly larger and heavier than females (marginally so for tail length), although the overall difference was relatively small: between 1.7-3.2% for all body measurements (Supplementary Table S3).

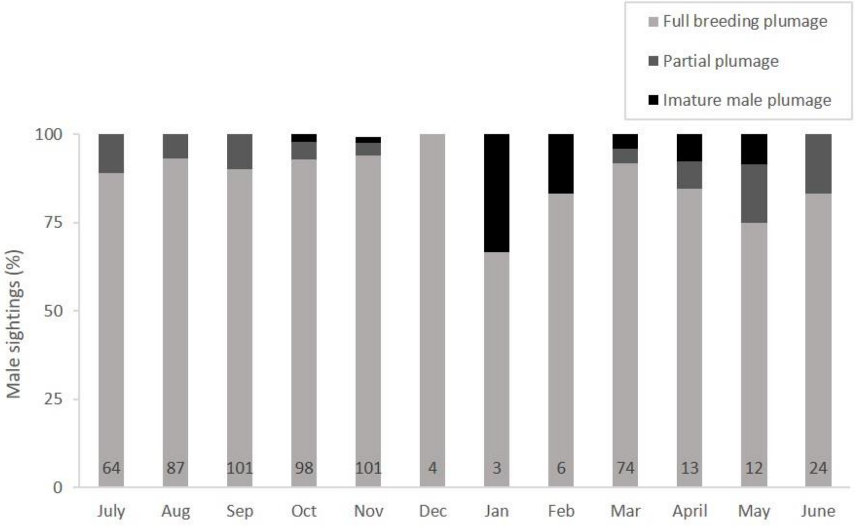

Figure 2. Seasonal variation in plumage status of banded males observed between 2013-2017. Numbers on the bottom at each bar indicates the total number of individuals observed each month in all years.

Behavioural displays

We observed diverse displays performed by male and female breeders and subordinates (details in Supplementary material and Table S4). Males mostly performed courtship displays to their female partner (83.3%), and seldom (16.6%) directed them to extra-pair females. Petal displays (carrying flower petal in bill; Supplementary Figure S6) were only performed by males and mostly in their own territory (69.7%), but also at the border and outside the territory (30.3%), mainly directed towards their own partner (24.2%), to simulated intruders (when luring birds to mistnets with playback of conspecific vocalisations, 39.4%), or intruding males (33.3%). In 27% of cases, the intruding male(s) also displayed a petal and both male and female of the intruded territory were present, so the receiver was unknown.

Male breeders sometimes fed females during nest building, incubating, or brooding (97.7% of ‘courtship’ feeds were by breeder, 2.3% by subordinate males).

Breeding activity

Active nests, dependent and independent juveniles were primarily seen between August and November (Figure 3), but nesting seemed to occur year-round, since dependent juveniles were also seen in March and July.

Most captured males (88 of 112) had a swollen cloacal protuberance (CP), including subordinate males. Males with no swelling of the CP included dominant males in full bright plumage with no active nest (from late May/early June 2015 *n* = 12), and males in moult or immature plumage (*n* = 12).

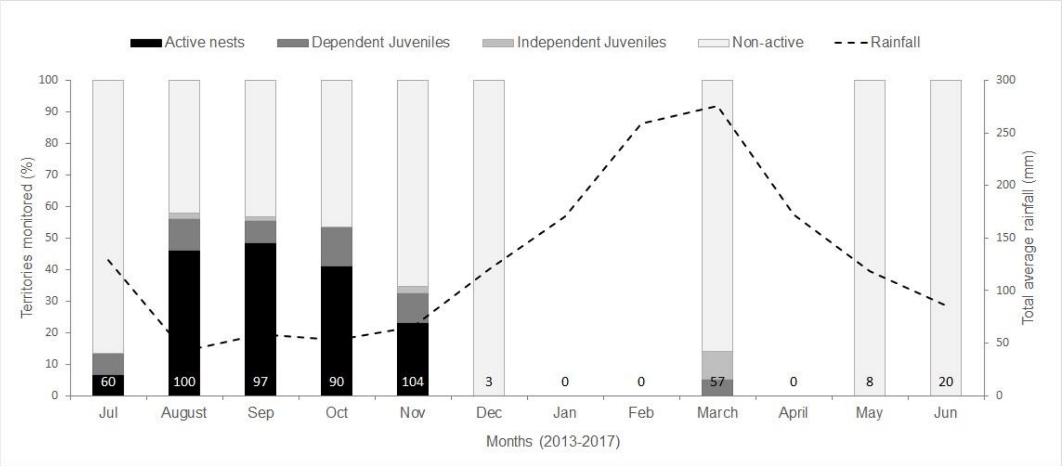

Figure 3. Seasonal variation in nesting activity, indicating the percentage of territories monitored with active nests, dependent juveniles, independent juveniles, or no active breeding, between 2013-2017. The dotted line indicates average between 2013-2017 of total monthly rainfall (rainfall data from Australian Bureau of Meteorology, Cairns station #031011). Numbers on the bottom at each bar indicates the total number of territories monitored each month in all years.

Nesting characteristics

Dominant females built the nests without assistance, taking on average 11 days (range 7-18 days; Supplementary Figure S7). Males were usually nearby during building, and on one occasion, a male was twice seen sitting in the nest that was being built.

Nests had a domed structure, of 421.3 ± 127 cc volume, 64 ± 96 cm height (range 10-700 cm), in different sites: suspended from tree branches and vines, *Pandanus*, small mangrove trees, mangrove ferns or grass (Supplementary Figure S7). Nests were often partially concealed (58% of 146 nests), or open (32%) and less frequently fully concealed (10%).

The first egg was laid one to seven days after nest completion, and further eggs were laid early in the morning on consecutive days. Eggs were moderately spotted, with greater concentration of dark-red spots on the blunt end of the egg (Supplementary Figure S7). Females laid clutches of two to three eggs (average in 2015: 2.2, n = 30; 2016: 2.6, n = 45, 2017: n = 13, 2.3), and on one occasion a female incubated only one egg. When eggs or nestlings were predated, females re-nested after one to four weeks. Between July and November, females initiated on average 2.1 nests (range 1-6 nests) and laid 1.9 clutches (1-4 clutches; n = 117).

Incubation typically started one day after the last egg was laid. Only females incubated, and occasionally males fed females in the nest. From captures, 61% of 98 females, and none of 111 males had a brood patch. Incubation lasted for on average 14 days (range 12 – 16 days). Bouts of incubation lasted from 20 min to over one hour. Eggs in the same clutch hatched on the same day. Hatching success was 89% (n = 23 nests).

Hatchlings were pink and naked, and their skin darkened within a day. On the third day primary pin feathers erupted. On the seventh and eighth day, the eyes opened, and the feathers started emerging (Supplementary Figure S8). Birds fledged in 12 to 14 days. Fledglings made continuous begging calls and were fed by group members for up to two months after fledging. Fledgling tails grew to full length within a month of leaving the nest.

Parental care and nest provisioning

All group members fed nestlings and fledglings. Only the female brooded the hatchlings, usually until the fifth day. Early in the nestling period, females brooded on average 23 minutes per hour (±9.68 min, *n* = 17). Feeding rate increased with nestling age (*F* = 4.089, *P* < 0.01; Figure 4), slightly more so by females (Age * Sex, *F* = 2.006, *P* = 0.09; Figure 4), but brood size did not influence feeding rate (*F* = 0.393, *P* = 0.68). Males and females fed at similar rates overall (respectively 4.5 ± 3.2 and 4.7 ± 2.6 feeds/h, *n* = 36, *F* = 0.208, *P* = 0.65). Nests with subordinates received more food than nests with just pairs (*F* = 7.104, *P* = 0.01): average 15.3 ± 6 feeds/h in total (*n* = 6 groups), 8.5 ± 4.8 feeds/h (*n* = 30 pairs).

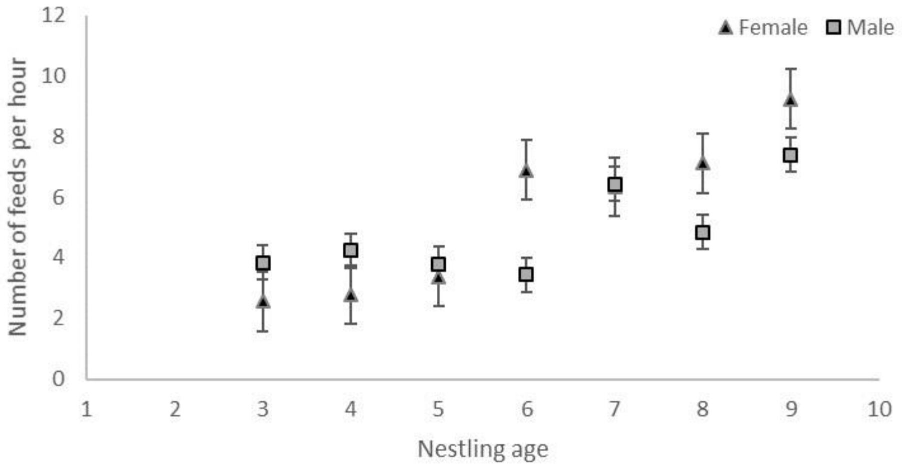

Figure 4. Feeding rate by female and male breeder as function of nestling age for days 3-9 (means ± SE).

Productivity and parasitism

Of the 70 nests found before laying was completed, 14% hatched but did not fledge any offspring. Including nests found after laying, success rates per stage were: 8% of 25 nests that hatched produced at least one fledgling, and 66% of nine nests that fledged had at least one fledgling surviving to independence. On average, 29% of monitored territories produced at least one surviving fledgling per year; pairs and groups with subordinates produced similar number of fledglings per year, respectively 0.6 and 0.5.

From 117 recorded nest failures, 15% were abandoned (during nest building or incubation), 83% were predated (59% during incubation, 23% with nestlings) and 2% were parasitised by a cuckoo. Camera traps identified the following nest predators: Lace Monitor *Varanus varius* (*n* = 4), unidentified species of Snake (*n* = 6), Black Butcherbird *Melloria quoyi* (*n* = 3), Brush Cuckoo *Cacomantis variolosus* (*n* = 1), and an unidentified species of Rodent (*n* = 1; Supplementary Figure S9). Except for the Brush Cuckoo and the Rodent that were only seen predating eggs and nestlings respectively, all other predator types were observed at both egg and nestling stage.

Two nests were parasitised by a Brush Cuckoo (Supplementary Figure S10). In both cases, an unassisted pair accepted and fed a cuckoo nestling. The first nest was found when the cuckoo nestling was ~eight days old. The second nest was found with three eggs (two LFW eggs and one cuckoo egg, Supplementary Figure S10), and after 13 days, the cuckoo nestling hatched (one day before the LFW) and evicted the eggs. Both nests failed due to predation of the cuckoo nestling (information retrieved from camera trap).

Discussion

The Lovely Fairy-wren shares many life-history attributes with its close taxonomic relatives. However, it also has characteristics consistent with expectations for tropical passerines, which may explain the life-history and behavioural differences in relation to other fairy-wrens (Table 1).

Population, social organisation, and survival

Consistent with previous observations that LFW occupy ecotones (Higgins et al., 2001), territories usually contained two or more plant communities differing in vegetation density. Territories were bounded by one or more neighbours and were maintained throughout the year, potentially facilitating opportunistic breeding year-round.

Lovely Fairy-wrens maintained long-term pair-bonds. Divorce rates while low (10.9% per year), were higher than for most other *Malurus* (Table 1). The causes of divorce are unknown but could involve females avoiding inbreeding when sons inherit the territory (28% of subordinate male LFW inherited their natal territory). In Superb Fairy-wrens *Malurus cyaneus* and Purple-crowned Fairy-wrens *Malurus coronatus* most divorces occurred when mothers divorced their sons (Cockburn, Osmond, Mulder, Green, & Double, 2003; Hidalgo Aranzamendi et al., 2016). Other reasons for divorce could be following low reproductive success, or to obtain a higher quality territory or partner (Choudhury, 1995; Hidalgo Aranzamendi et al., 2016).

Long-term pair-bonds were associated with high adult breeder survival (86%), which was generally higher in LFW than in other *Malurus* species (Table 1). High adult survival could be associated with environment type, with dense habitat potentially decreasing the likelihood of adult predation (Medina et al., 2017). It could also be related with low risk-taking behaviour, as tropical birds appear to be risk-averse (Møller & Liang, 2013), and may not need to take risks and forage at higher rates, if resources are more stable in less seasonal environments.

Like other fairy-wrens LFW are cooperative breeders (Margraf & Cockburn, 2013), and the recruitment of subordinates occurred through natal philopatry. Still, only one-third of monitored groups had subordinates, and the size of the group in this species was lower compared to some other fairy-wrens (Table 1). Small group sizes could be a consequence of low productivity, or abundant habitat and breeding vacancies for dispersal of subordinates. In the Red-winged Fairy-wren *Malurus elegans*, low turn-over, adult mortality, and the rarity of vacancies for breeders resulted in large groups with many subordinates including females (Rowley & Russell, 1997).

In all fairy-wrens so far studied, populations are male-biased, probably because of high female mortality associated with natal dispersal (Margraf & Cockburn, 2013). Our study populations were also male-biased, and females dispersed earlier and farther from natal territories than males. Moreover, no female subordinates were seen on territories with active nests, while 95% of male progeny stayed in their natal territory for at least one year, assisting their group with offspring provisioning throughout the breeding season. Observations in captivity of LFW showed that immature females, but not males, were killed by breeding adults (Schodde, 1982), suggesting that first-year females may be forced to disperse when sexual maturity is reached.

Groups with subordinates had similar numbers of fledglings than unassisted pairs. While we found no evidence for any direct benefit of subordinates via productivity, they may compensate through load lightening, and reduce costs of reproduction (Russell, Langmore, Cockburn, Astheimer, & Kilner, 2007; Tidemann, 1986), or by increased care, which can increase offspring growth and survival. In agreement with the latter, we observed higher total provisioning rates in groups compared to unassisted pairs.

Female and male traits

LFW are sexually size-dimorphic, with males slightly larger than females, as in other fairy-wrens (Rathburn & Montgomerie, 2003; Rowley & Russell, 1997). The only non-significant morphometric difference between sexes was tail length, and although some species of fairy-wrens show reversed sexual dimorphism in tail length, which is suggested to be a selected ornamental trait in birds (Swaddle, Karubian, & Pruett-Jones, 2000), this is not the case for the LFW.

Fairy-wrens are also strikingly variable in plumage dichromatism, due to directional selection on both female and male plumage (Johnson et al., 2013; Karubian, 2013). It has been proposed that for male fairy-wrens, sexual selection has driven the evolution of bright plumage (Johnson, *et al*. 2013, Friedman and Remeš 2015). In females, plumage colouration follows a latitudinal gradient of cryptic colours in the south and colourful plumages in the north of Australia and New Guinea, except for Red-backed fairy-wrens *Malurus melanocephalus* (Friedman & Remeš, 2015; Johnson et al., 2013). Recent work suggests that this latitudinal pattern is driven by more colourful plumaged females occupying denser vegetation while dull-plumaged females occupy open habitat (Medina et al., 2017). Ecological factors might have shaped ornament expression but is unlikely that this is the sole determinant, and variation in life-history traits may be associated with colouration in the LFW. The extended breeding period, low territory turnover, high adult survival and great female dispersal suggests that competition for partners or territories may be intense. For example, plumage colour may regulate defence of territories year-round and deter other competitors (Tobias, Montgomerie, & Lyon, 2012), but further experiments are required to test for this.

There has been extensive research into sexually selected functions of the bright plumage sported by male fairy-wrens during the breeding season (reviewed in Peters et al., 2013). Most male fairy-wrens moult annually between a bright breeding plumage, and a dull winter (eclipse) plumage. Timing for both the initial and subsequent seasonal acquisition of bright plumage is highly variable and influenced by age, testosterone, social status, and condition (Peters et al., 2013). Surprisingly, we found that the bright plumage of male LFW is not a seasonal breeding plumage, but rather an adult plumage acquired within a year of hatching and retained thereafter by both breeder and subordinate males. The duration of moult into bright plumage took several months, much slower than other fairy-wrens (Table 1; e.g. Superb Fairy-wren: 25 days; Mulder & Magrath, 1994). The absence of a seasonal moult between bright and dull plumages is common in New Guinea fairy-wrens (*M. cyanocephalus and M. alboscapulatus*, Table 1) but seems to be unique to LFW among male Australian fairy-wrens (Peters et al., 2013; Table 1), although subspecies *M. lamberti rogersi* and *M. l. dulcis*, which also have somewhat colourfully plumaged females, remain to be studied. In female LFW, adult bright plumage is likely to be acquired while still in the natal territory, and while the duration of moult is unknown, like males, after attaining bright adult plumage females maintain it year-round.

The nature of plumage acquisition in male and female LFW challenges the generality of theories around seasonal plumage in fairy-wrens. If bright plumage has a breeding or territorial function, it may be maintained because LFW breed year-round. However, Purple-crowned Fairy-wrens, breed and defend territories year-round, yet adults have seasonal plumages (Fan et al., 2017; Hall & Peters, 2009; Rowley & Russell, 1997). Since seasonal loss of bright plumage in males appears to be associated with dull female plumage across fairy-wren species, a non-mutually exclusive explanation is that predation risk selects for seasonally cryptic plumage in males and dull plumage in females. In Superb Fairy-wrens, bright males have higher perceived risk of predation compared to dull males and females (McQueen et al., 2017), although in Red-backed Fairy-wrens survival rates do not differ between dull and bright plumages (Karubian et al., 2008). An experiment using 3D fairy-wren models showed that probability of predator attacks is higher in open than in closed habitats (Cain et al., 2019), suggesting that the dense tropical habitats of LFW may reduce their exposure to predators, compared to species in open habitats. Overall, the function of seasonal plumage remains enigmatic, and testing the function and fitness consequences of colourful plumage in the LFW will be an important next step in our understanding of the evolution of ornaments.

Breeding behaviour and life history traits

LFW breed intermittently throughout the year, with a preference for the dry season. Benign temperatures throughout the year, and less variable breeding conditions may allow for longer breeding seasons (Wyndham, 1986). Species living in tropical regions typically also exhibit 'slow' pace of life (Wiersma, Muñoz-Garcia, Walker, & Williams, 2007), including most of the Australian avifauna (Bech, Chappell, Astheimer, Londoño, & Buttemer, 2016; Russell et al., 2004). Nonetheless, compared to other fairy-wrens (Table 1), these characteristics are more pronounced in LFW: high adult survival, slow juvenile maturation, longer dependence (for 2 months) and low reproductive success.

In fairy-wrens, the main causes of nest failure are predation, brood parasitism, and desertion (from highest to lowest impact; Rowley & Russell, 1997). In LFW, predation is the main driver of low productivity, consistent with high levels of nest predation for animals living in tropical Australia (Remeš, Matysioková, & Cockburn, 2012). Brood parasitism has a significant impact in other malurids (Rowley & Russell, 1997), but although this study adds two further cases to the recent first report of parasitism of LFW (De Geest & Leitão, 2017), parasitism was nevertheless a minor cause of nest failure.

All fairy-wrens are socially monogamous, though they also have among the highest rates of extra-pair fertilisations in birds. For example, in Superb Fairy-wrens, 76% offspring are fathered by an extra-pair mate (Mulder et al., 1994), and 96% of courtship displays are performed to extra-pair females (Mulder, 1997). In contrast, LFW male courtship displays were mainly directed towards their partner (83%). Also, all *Malurus* males, except Purple-crowned Fairy-wrens, perform petal displays thought to be linked to high rates of extra-pair mating (Karubian & Alvarado, 2003; Mulder, 1997; Rowley & Russell, 1997), but in the LFW these displays usually occurred in their own territories towards a partner or in response to a territorial intrusion. In some cases, intruding males also displayed a petal when both male and female of the intruded territory were present, so the behaviour could have been directed towards the extra-pair female. In Superb Fairy-wrens, 97% of the petal displays were directed towards extra-group females (Mulder, 1997), and in Red-backed Fairy-wrens 90% of displays were towards females and 50% of these were performed outside their own territory (Karubian & Alvarado, 2003). In both species, the primary function of petal displays is inter-sexual and extra-pair attraction, whereas in the LFW petal displays are likely to function in both intra and inter-sexual contexts.

Males invest little in parental care in some fairy-wren species, suggested to be associated with high extra-pair paternity (Møller, 2000; Mulder et al., 1994). Both female and male LFW cared for nestlings and juveniles, and the dominant male made as many feeding visits to the nest as the female (though sexes can vary their investment in relation to food size, see Colombelli-Négrel & Kleindorfer, 2010), contrasting with some other fairy-wrens, where females provisioned at higher rates than males (Brouwer et al., 2017). In LFW, male and female coordination might be necessary to facilitate the extended parental investment.

The reduced frequency of courtship displays outside the territory, high male care, low bird density, and reduced number of subordinates, together suggest that in this species extra-pair mating may occur at lower frequencies (Brouwer et al., 2017). Further study focusing of the genetic mating system of the LFW may illuminate the traits and behaviours associated with extra-pair behaviour.

Conclusions

Our study contributes to understanding the biology and natural history of the LFW, the diversity of the *Malurus* genus, and addresses a scarcity of studies of tropical birds. The findings here also provide important background information which will allow the investigation on the functional significance of behavioural displays and bright plumage, to better understand the processes behind the evolution and expression of female and male displays and ornamentation.

Acknowledgments

We are grateful to Rachel Shepperd, Gaia Marini, Philip Chaon, Irene Cuesta, Zoe Zelazny, Luke Nelson and Kelsey Bell for their field assistance, and Kristal Cain, Phillipa Cannon, Patrick De Geest, Lucy Friend, and local birders for their support. We thank Lesley Brooker for identifying the cuckoo nestling and Caterina Funghi, Inês Leitão, Kate Buchanan, associate editor, and anonymous referees for helpful comments on the manuscript. We acknowledge Cairns Council and Cairns Airport for giving access to the study sites. This research was supported by the University of Melbourne and grants from the Australian & Pacific Science Foundation (APSF1406), the Stuart Leslie Bird Research Award (Birdlife Australia), the Australian Research Council (DP150101652), as well as a kind donation from the Bolton family in memory of Dick Bolton's love for birds.

Appendix

Methods

Molecular sexing

Nestlings, fledglings, and adults that could not be sexed based on plumage characteristics or behavioural observations were sexed using PCR-methods (*n* = 29) described in detail in Griffiths *et al.* (1998). DNA was extracted from blood samples using the standard salt extraction method (Bruford, Hanotte, Brookfield, & Burke, 1992). We used known sexed individuals as controls.

Nesting behaviour

Observations at different stages during the nesting cycle were used to estimate key nesting parameters (first egg date, hatch date, etc.). Estimates were based on subsets of nests checked every day to three days, to accurately calculate the following periods: duration of construction (from early frame until lining was complete and female was not seen building), laying period (between the first egg and the last egg laid), incubation (between the last egg being laid and the female seen sitting until eggs hatched), nestling period (between hatching and fledgling). Once the timing of nesting stages was known, nest checks occurred during period-changing events (e.g. laying, hatching, fledging). When the attempt was found at later stages, we estimated the date the first egg was laid by calculating back from the hatch date; if found with nestlings, we estimated their age by the length of primary wing feathers. We recorded the outcome for each nest found –abandoned or destroyed, parasitised, predated and at which stage, and fledgling survival.

Nestling development was followed by taking note of colour of the skin, emerging of feathers, and opening of eyes. Between day 7 and 9 after hatching, we took morphometric measurements, a small blood sample and colour-banded the nestlings. We estimated fledgling survival by checking the nest and then observed the group for presence of fledglings. Fledgling age was estimated by the tail length (juveniles one month after fledging or younger had shorter tails than adults), and/or behaviour (fledglings called constantly and displayed begging behaviour). We calculated productivity and reproductive success as the number of nests in which at least one nestling fledged and survived for more than a month, considering all the nests found across seasons (2015-2017).

Description of behaviours recorded during focal watches

Aggressive displays were performed by males, females and subordinates and consisted of individuals opening wings and erecting feathers, rapid flights. singing, production of loud calls bill snapping, and chasing intruders.

Courtship displays were very brief and given usually by males in the presence of their females, and consisted of males erecting their blue back feathers, the red shoulders, and the blue of the ears, combining with very rapid flights and the male chasing the female.

Petal displays were performed by males only (Figure S6) and consisted of displaying in their bills, petals of different sizes and colours (red and yellow) combined it with flights and sometimes chasing and aggressive behaviour, depending on the context.

Male courtship feeding was mainly observed towards their partner and sometimes in response to female wing-fluttering display. On two occasions, a dominant male was seen feeding a subordinate male (in full adult plumage), but never the other way-around.

Copulation was seen on three occasions always in the morning: at 11.05am on the 27/08/2015, just after the female was seen building and a male did courtship display; at 07.28am on the 22/07/2016, after the dominant male was observed actively singing and the female performed a wing fluttering display (a week after a complete nest with no eggs was found); the third event occurred at 09.10 am on the 20/08/2017, after the female was seen actively building a nest.

Figures

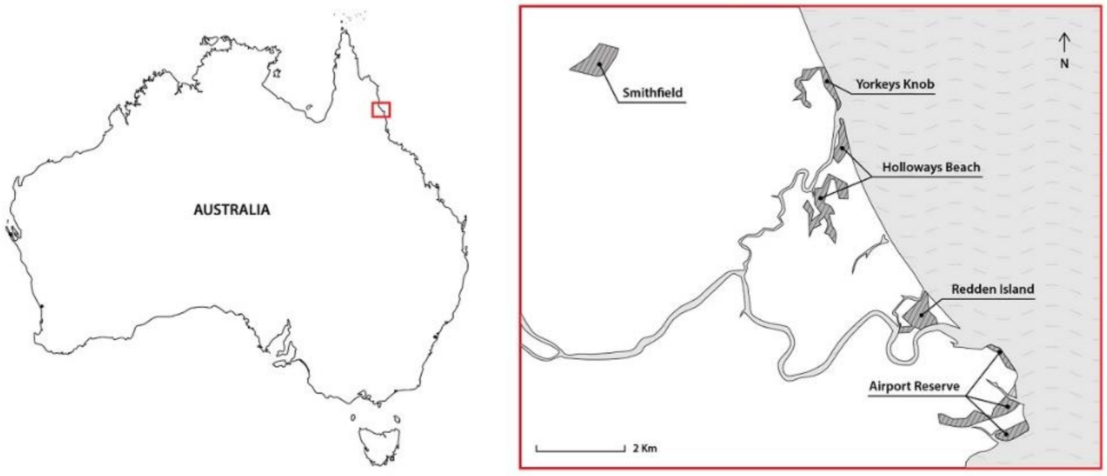

Figure S1. Study areas in north of Cairns, Queensland, Australia. Shaded lined areas show the location of all areas where territories were monitored.

Figure S2. Habitat within a territory where Lovely fairy-wren *Malurus amabilis* nests where found. Photos taken in the Airport Reserve, Redden Island and Yorkeys Knob, Cairns, Australia.

Figure S3. Patterns of male moult from juvenile to adult bright plumage. From top left to right: male with juvenile plumage; 5 % moult; 30 % moult; 60 % moult; the two photos in the bottom row show the same two males, in dorsal and side views, respectively in 70 % moult 100 % adult bright plumage.

Figure S4. Female plumage of xM/xan. a) First captured as juvenile in the natal territory, with dull plumage, on 14 Sep 2015 b) Re-capture as adult in the breeder territory, with bright plumage, on 11 Sep 2017.

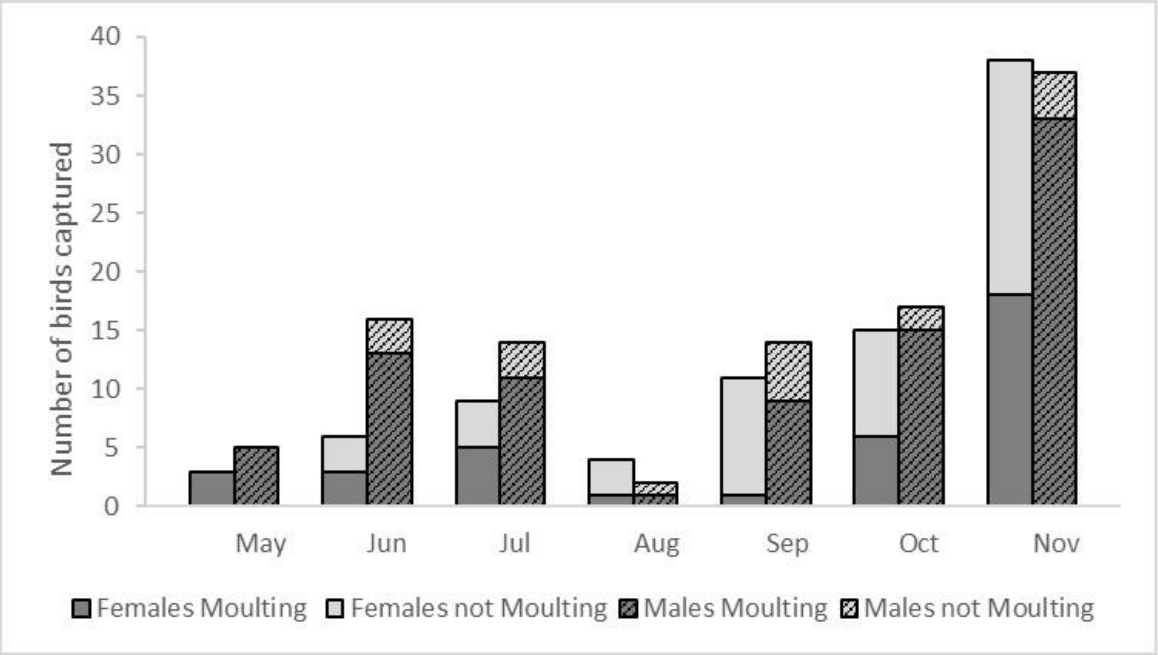

Figure S5. Seasonal variation in body moult of individuals captured between 2013 and 2017. Bars show, for each month, the number of different males (hatched) and females (clear) captured that were moulting (pin feathers present, dark grey) or had no sign of body moult (light grey).

Figure S6. Male Lovely fairy-wren performing a petal display. Credit: Patrick De Geest.

Figure S7. Breeding activity. From top left to right: Nests medium frame; complete nest; female building early frame nest, credit: Kristal Cain; complete nest with two eggs laid; female incubating. Credit: Philip Chaon.

Figure S8. Lovely fairy-wren nestlings from different breeding attempts. From top left to right, nestlings Day 2, Day 3, Day 5, Day 7 (hatch day = Day 1).

Figure S9. Nest predation events by Black Butcherbird *Melloria quoyi*, Lace Monitor *Varanus varius*, Brush Cuckoo *Cacomantis variolosus*, unidentified snake (photographs taken with Bushnell Trophy Cam HD Aggressor).

Figure S10. Brood parasitism by Brush cuckoo. From top left to right, 3 eggs (cuckoo egg in the middle), nestling Brush Cuckoo *Cacomantis variolosus* (species identity confirmed by L. Brooker pers. comm.) Day 2, Day 3, Day 6, Day 9 and possibly Day 11. The first 5 images are from the second attempt found with parasitism where we followed all stages, and the sixth photo is from the first attempt found with a nestling, so age is an estimate based on the growth of feathers.

Tables

Table S1. Ethogram of behaviours recorded

Behaviour	**Description**
Feed nestling or juvenile	Frequency that birds are seen carrying food to nest, or feeding juveniles that are begging
Lost focal individual	Focal individual is not seen but behavioural watch is still being conducted
Wing flutter	Number of times individual flaps the wings quickly and does a begging behaviour, usually females
Courtship feed	Focal individual feeds another individual
Courtship display	Focal individual (male) is seen chasing female and erecting cheek, crown, and shoulder feathers
Copulation	When mating is seen
Petal display	Frequency and duration a focal individual (male) is seen carrying a petal. Also recorded the colour of the petal and context in which it was observed – other males around, intrusion, towards females, alone.
Carrying nesting material	Number of times female is seen carrying nesting material.
Building nest	Number of times female is seen building nest
Sitting on the nest	Occurrence: when the nest is being observed and female is sitting on it.
Aggression display	Frequency and duration of aggression display – loud calls, feathers erected, rapid movements and physical contact, chasing. Context of aggression is registered
Intrusion on territory	When focal individuals intrude a territory or focal individuals are intrude by other(s).

Table S2. Annual survival (%) of female and male adults (dominant/breeders), and sample size.

	Females	**Males**	**Total**
2014	100% (7)	100% (9)	100% (16)
2015	100% (10)	93.3% (14)	96.6% (24)
2016	76.3% (29)	85.3% (35)	80.8% (64)
2017	61.5% (24)	71.4% (30)	66.4% (54)

Table S3. Morphological differences between the sexes (mean ± sd (sample size)). Sexual size dimorphism was calculated as 100 * ((Male - Female) / (Male + Female) / 2). P values represent significance Mann-Whitney tests of male and female parameters.

	Female	**Male**	**Average size dimorphism (%)**	**Sex difference**
Body mass (g)	8.52 ± 0.84 (75)	8.76 ± 0.46 (84)	2.8%	$P < 0.001$
Tarsus length (mm)	20.82 ± 0.67 (76)	21.34 ± 0.63 (83)	2.5%	$P < 0.001$
Head–bill length (mm)	28.98 ± 1.44 (76)	29.83 ± 0.73 (83)	2.8%	$P < 0.001$
Wing length (mm)	49.26 ± 1.33 (75)	50.86 ± 1.52 (83)	3.2%	$P < 0.001$
Tail length (mm)	56.99 ± 4.03 (68)	57.97 ± 3.47 (78)	1.7%	$P = 0.054$

Table S4. Breeding and related behaviours from dominant/breeder male (Dom Male), dominant/breeder female (Dom Fem.) and subordinate male (Sub Male), regarding context, location, and display receiver (Directed towards). Number of observed behaviours in parenthesis.

Behav.	*Actor*	*Context**	*Location*			*Directed towards*				
			In territory	*Outside*	*Border*	*Dom Fem*	*Male intruder*	*Female extra-pair*	*Alone*	*Play-back*
Court. display	Dom Male	U; BN; NB; L; N; NF	83.3% (15)	16.6% (3)	-	83.3% (15)	-	16.6% (3)	-	-
Petal display		U; TD; NB	69.7% (23)	9.1% (3)	21.2% (7)	24.2% (8)	30.3% (10)	?	3.0% (1)	33.3% (11)
	Sub Male					-	3.0% (1)	?	-	6.1% (2)
Feed.	Dom Male	NB; I; B	100% (87)	-	-	97.7% (85)	-	-	-	-
	Sub Male					2.3% (2)	-	-	-	-

* U – Unknown; TD - Territorial dispute; BN- Before nest building; NB – Nest building;
L- Laying; N- Nestlings; I -Incubating; B – Brooding; FN- Feeding nestlings; NF – Nest failure.

Chapter 3

Female and male plumage colour signals aggression in a dichromatic tropical songbird

Ana V. Leitão, Michelle L. Hall, Kaspar Delhey, Raoul Mulder

Animal Behaviour (in press)

Abstract

Animal signals of competitive ability allow contests for limited resources to be settled without costly physical fights. Traits indicating competitive ability are diverse and span visual, acoustic, or chemical modalities. Although animal signalling has been intensively studied, research has focused mainly on male traits. Little is known about the extent and functionality of competitive signals in females and whether there are sex differences in signal function. We studied whether plumage colour signals competitive ability in female and male lovely fairy-wrens, *Malurus amabilis.* In this species, both sexes sport elaborate but sexually dichromatic ornamental plumage. Using a mirror image stimulation test, we first assessed the relationship between male and female colour and agonistic behaviour, controlling for other physical, social, and ecological variables. We then tested whether colourful plumage influenced aggressive response in both sexes by experimentally manipulating plumage colour and measuring individual responses to their mirror image. Females and males were more aggressive towards naturally less colourful reflections of the cheek patch in the mirror. However, when we manipulated plumage colour, both females and males responded more aggressively to experimentally increased cheek colour reflection in the mirror. Our findings suggest that plumage colour signals competitive ability in an aggressive context in both sexes and raises the possibility that signal reliability may be maintained by social interactions where individuals police and punish dishonest signals.

Keywords: badge of status, colour manipulation, competitive signals, honest signalling, *Malurus*, mirror test, social cost, social punishment.

Introduction

Competition for limited resources is a vital component of survival and reproduction and a major selective force. Historically, most studies have focused on male–male competition (Andersson, 1994), although females also compete for sexual and nonsexual resources (e.g. food, territories), in a range of taxa including reptiles (While, Sinn, & Wapstra, 2009), fish (Forsgren, Amundsen, Borg, & Bjelvenmark, 2004), insects (Papadopoulos, Carey, Liedo, Mulle, & Sentuerk, 2009), birds (Cain & Langmore, 2015; Pryke, 2007), and mammals (Holekamp, Smale, & Szykman, 1996), including humans (Vaillancourt, 2013). In females, aggressive interactions can help improve conditions for breeding (Rosvall, 2008), access to high-quality reproductive resources (Sandell & Smith, 1997) or generally increase reproductive success (Sinn, While, & Wapstra, 2008).

Traits such as colourful plumage or elaborate vocalizations (hereafter ‘ornamental traits’) can be signals of social status that help settle contests by honestly informing rivals about the costs of escalating aggression (status signalling theory; Rohwer, 1975; Senar, 2006). Males and females of many species may display such competitive trait signals (Tobias et al., 2012) that are driven by selection acting differentially between sexes (Dale et al., 2015), but while these are generally well studied in males, our understanding of female signals is still limited (Rosvall, 2011). It has been hypothesized that female ornamental traits are maintained as inherited non-functional by-products of selection on male ornaments (genetic correlation: Lande, 1980) or shaped by natural selection (Bortolotti, 2006; Wallace, 1895). Alternatively, female ornamental traits may be maintained by direct selection, as sexual signals (Amundsen, 2000a) or generally as social signals for competition over sexual and nonsexual resources (Tobias et al., 2012; West-Eberhard, 1979). Female ornamental traits may or may not be shaped by the same selective processes as in males (Clutton-Brock, 2007; LeBas, 2006). Thus, to understand and address the different hypotheses for female ornament evolution, we must evaluate the possible function(s) of female ornaments and directly compare them to those of males. So far, a few studies have contrasted female and male ornaments in same-sex competition and intersexual differences in signal function (Crowhurst, Zanollo, Griggio, Robertson, & Kleindorfer, 2012; Mónus, Liker, Pénzes, & Barta, 2016; Murphy et al., 2014; Reedy et al., 2017; van Dongen & Mulder, 2008; Young, Cain, Svedin, Backwell, & Pryke, 2017).

Generalized insights about ornamental trait function, such as plumage coloration, have been drawn primarily from studies of temperate species. This is problematic because tropical regions have the greatest species diversity (Stutchbury & Morton, 2001), and in the tropics males and especially females are more colourful than in temperate environments (Dale et al., 2015). Therefore, selective pressures on colourful plumages might vary between temperate and tropical regions.

The objective of this study was to investigate whether plumage colour functions as a competitive signal in both sexes of the lovely fairy-wren, *Malurus amabilis*, a resident tropical passerine with year-round territories and reproduction. The lovely fairy-wren provides an opportunity to investigate possible functional aspects of coloration, since males and females are both colourful but sexually dichromatic, a good criterion for studying competitive traits (Amundsen & Pärn, 2006). Furthermore, within the Australian fairy-wrens, female lovely fairy-wrens express the most colourful plumage, and males, in contrast to most Australian fairy-wrens, do not lose their colourful plumage outside the breeding season (Leitão et al., 2019). Additionally, lovely fairy-wrens have tropical life history traits, such as year-round breeding and territorial defence (Leitão et al., 2019), which may offer a contrasting view on the evolution and function of ornaments compared to well-studied temperate species.

We used mirror image stimulation to experimentally assess same-sex competition. An individual's reaction to its own reflection in a mirror was interpreted as its response to a perceived conspecific or rival, since birds typically do not recognize their mirror image (e.g. New Caledonian crows, *Corvus macrorhynchos*: (Medina, Taylor, Hunt, & Gray, 2011); jackdaws, *Corvus monedula*: (Soler, Pérez-Contreras, & Peralta-Sánchez, 2014); great tits, *Parus major* (Kraft, Forštová, Urhan, Exnerová, & Brodin, 2017); the sole exception is the Eurasian magpie, *Pica*: (Prior, Schwarz, & Güntürkün, 2008)). Mirrors have been used to study aggression in a range of species, such as crayfish (May & Mercier, 2007), fish (Oliveira, Carneiro, & Canário, 2005; Wilson, de Boer, Arnott, & Grimmer, 2011), birds (Branch, Kozlovsky, & Pravosudov, 2015; Hirschenhauser, Wittek, Johnston, & Möstl, 2008) and mammals (Svendsen & Armitage, 1973). Mirror image stimulation provides an opportunity to understand how individuals perceive physical traits such as plumage coloration since it has the benefit of standardizing the opponent's behaviour (to 'mirror' the subject), remove confounding effects by testing in social isolation and excluding all stimuli (acoustic, tactile and chemical) except for visual ones (Gallup Jr, 1968; Ruzzante, 1992).

We aimed to investigate whether male and female plumage colours in lovely fairy-wrens (1) are a signal and (2) function in a competitive context. We tested whether: (1) the degree of aggression birds expressed when exposed to mirror image stimulation was related to plumage colour; (2) experimental manipulations of plumage colour changed the aggressive response of birds subjected to mirror image stimulation; and (3) males and females behaved differently in response to mirror image stimulation. We predicted first that if plumage coloration signals competitive ability, birds would behave more aggressively to unmanipulated ‘rivals’ perceived to be less colourful (and thus less intimidating). If increased coloration reflects the degree of threat or challenge, the reverse expectation (higher aggression towards a more threatening signal) might be expected (see Searcy, Anderson, & Nowicki, 2008). Second, if aggression is an intrinsic state related to the individual’s plumage state or colour, manipulation should have no effect, and individuals’ responses to a mirror image will be similar to those in the unmanipulated condition. Alternatively, if elaborate plumage colour signals status, individuals with enhanced colour should be less aggressive towards the more colourful ‘rival’ reflected image. Finally, if females and males have similar roles in competition, both should show similar patterns between plumage coloration and aggression.

Methods

Study species and field data collection

The lovely fairy-wren is a small nonmigratory bird, endemic to the wet tropics of Australia that breeds throughout the year, but primarily in the dry season (Leitão et al., 2019). It is a facultative cooperative breeder that forms long-term pair bonds and maintains territories year-round. Resident males, females and subordinates engage in coordinated territorial disputes that can escalate to physical aggression between same-sex opponents (Leitão et al., 2019). Adult males and females are colourful but dichromatic (Appendix Fig. A1); fledglings of both sexes have a duller version of female-like plumage. Males and females may take up to several months to moult to their adult plumage, both maintaining bright plumage year round thereafter (no seasonal change in plumage colour: Leitão et al., 2019).

We studied colour-banded lovely fairy-wrens in the Cairns region, North Queensland, Australia in 2013–2017. Adults and juveniles were captured in mist-nets (lured with playback calls) for banding and collecting morphometric measurements as well as for colour measurements and the mirror image stimulation test (described below). Birds were marked with a numbered metal band (Australian Bird and Bat Banding Scheme) and a unique colour combination. We measured morphological traits, including tarsus, wing, and head–bill lengths (vernier calliper, ±0.05 mm).

After release, birds were monitored weekly for several months a year, and social status, nesting behaviour, bird density and territory size were recorded (details in Leitão et al., 2019).

Mirror image stimulation test

During the dry seasons of 2015 and 2016, when birds were captured, we performed a mirror image stimulation test, close to the capture location in a modified bird cage (60 x 30 cm and 30 cm high; Fig. 1), with a mirror (30 x 30 cm) on the side wall of the cage concealed by a removable plastic cover.

We conducted two experiments. In experiment 1, (28 May to 17 November 2015), we assessed aggressive behaviour of females and males in response to mirror image stimulation, using birds with unmanipulated plumage colour (N = 23 females and 19 males). We excluded tests of subordinates (N = 4), to control for social status. In experiment 2 (25 July to 23 November 2016), we experimentally manipulated the colour of the cheek patch in both sexes (the only variable significantly related to aggression in experiment 1, see Results), and again recorded aggressive responses to mirror image stimulation (total for all treatments N = 35 females and 29 males, see below). We excluded from the analysis subordinates (N = 5), juveniles (*N* = 2), males that were moulting (*N* = 5) and individuals whose spectral files were lost (*N* = 2).

Cheek colour was manipulated in experiment 2 using three different treatments of water-based nontoxic colour marker pens (Ecoline brush pens, Royal Talens, Apeldoorn, Netherlands). We tested different colour pens on feather samples and measured their reflectance. The resultant reflectance spectra were then compared with the natural plumage spectra of the individuals, to select the best match for each treatment. In treatment 1 we used a 'blender' (uncoloured) pen intended as a control; in treatment 2 we used 'warm grey' pen colour (hereafter 'grey') intended to decrease coloration; and in treatment 3 we used 'ultramarine blue deep' pen colour (hereafter 'blue') intended to enhance blue coloration (treatment effect is shown in Fig. 2). Overall, treatment 1 'blender' maintained coloration as intended, but both treatment 2 'grey' and treatment 3 'blue' increased coloration (Appendix Table A1). Most treatments fell within the natural variation (Appendix Tables A1-A2), although in some cases the blue treatment increased coloration outside the observed natural range. For this reason, we consider treatment 1 'blender' as maintaining colour, treatment 2 'grey' as increasing colour within the natural variation and treatment 3 'blue' as increasing colour within and beyond the natural variation (for more details see Appendix, Experiment 2: Treatment effect). In total, we tested 10 'blender', eight 'blue', 11 'grey' males and 12 'blender', 10 'blue', 13 'grey' females in adult plumage (*N* = 35 females and 29 males). Treatment reduced the existing correlation between cheek colour, shoulder, and body size, but cheek colour was correlated in a similar manner with other blue patches (Appendix Table A3).

Behaviour in the mirror test was recorded with a video camera (GoPro HD Hero2, GoPro Inc., San Mateo, CA, U.S.A.) for 10–20 min. In the first half of the test the mirror was covered;

and in second half it was exposed by pulling the plastic cover from a distance. Most trials were conducted between 0600 and 1300 hours, local time (GMT +10), although some captures were also made in the afternoon (1500–1800 hours). From the video recordings, we analysed time spent in each area of the cage relative to the mirror (close to the mirror, neutral or far; Fig. 1; Supplementary Video S1) and aggressive behaviours, that is, the total number of displays (feathers erected, wings extended, usually performed at neutral distance), pecks and swoops (physical contact) towards the mirror (Supplementary Video S1). Since aggressive behaviours were all correlated ($r_\rho > 0.22$, $P < 0.02$), we combined them in a single score (sum of all aggressive displays). All behaviours were quantified separately for the periods with the mirror covered and the mirror uncovered. Aggressive behaviours were only observed after the mirror was exposed and were similar to aggressive behaviours observed in the field (Leitão et al., 2019).

We obtained high-quality sound recordings from the mirror test experiments (in 2015 recorder Marantz PMD660 with a microphone Sennheiser ME67, in 2016 recorder ZOOM H5 XYH-5 with a microphone Olympus ME-15). We used spectrograms generated in Avisoft-SASLab Pro v5.2.09 (Avisoft Bioacoustics, Berlin, Germany) to count the songs (‘reels’: Rowley & Russell, 1997) from females and males and analysed song rate (songs/min) from before and after the mirror was exposed.

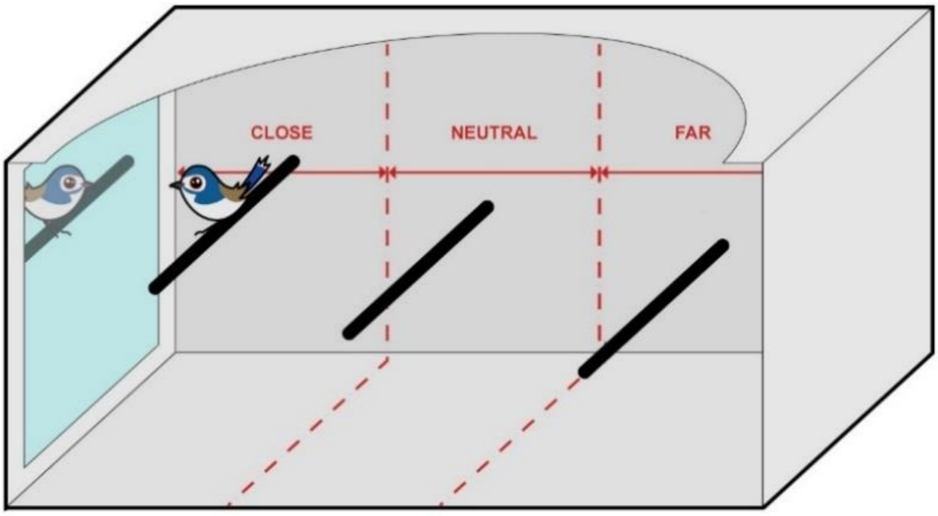

Figure 1. Diagrammatic front view of the mirror image test cage. The cage had three perches at different heights. One of the ends of the cage was covered by a removable plastic board covering a mirror, and on the opposite side birds were introduced through a small hole that was then covered. The mirror was uncovered (from a distance) approximately 5 min after the bird entered the cage. The walls of the cage were white, and the front was covered with wire to allow illumination and video recording. To minimize external influence, a white cloth wall covered the camera and the wired part of the cage. We analysed time spent in each area of the cage relative to the mirror (close, neutral, or far). Image reproduced, with permission, from Osvaldo Branquinho.

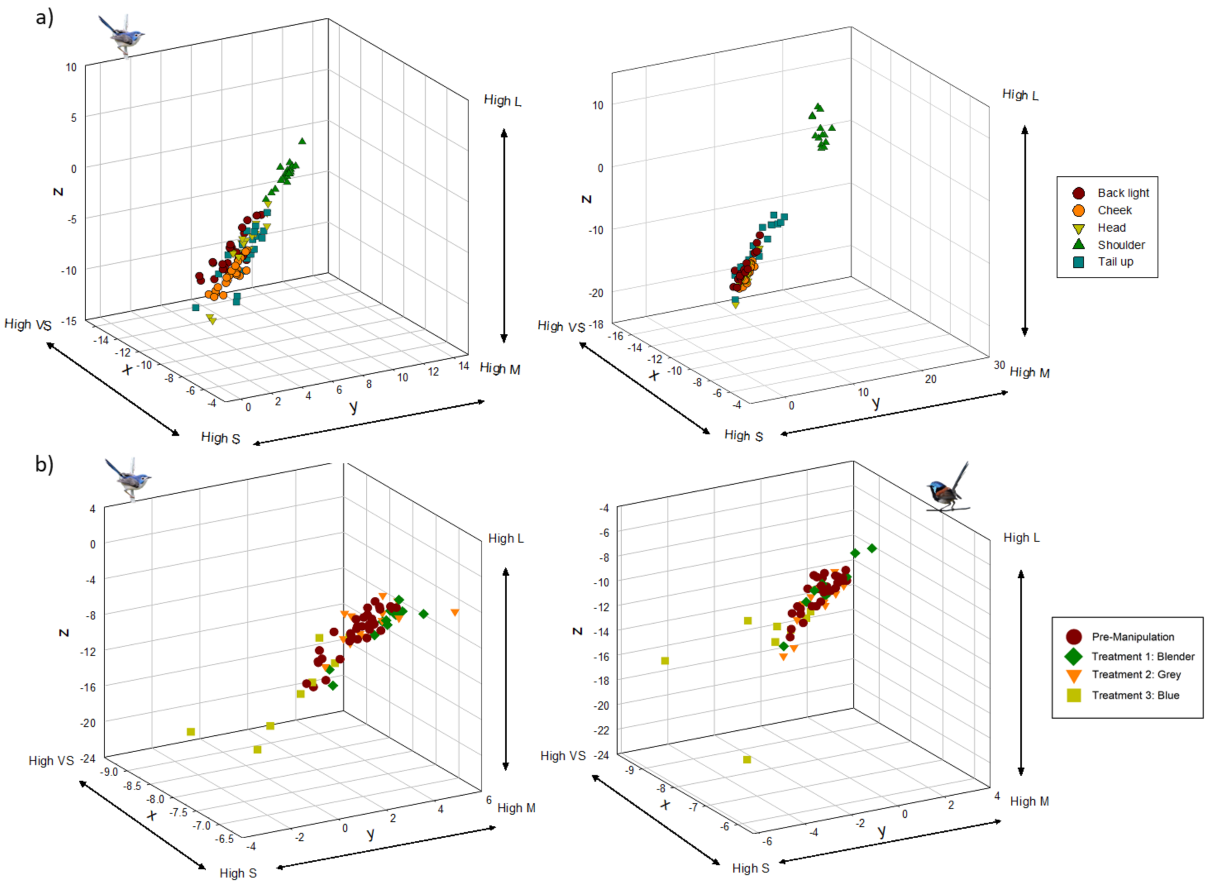

Figure 2. Graphic representation of chromatic coordinates (xyz) plotted in the visual space of U-type (ultraviolet-sensitive) birds, for plumage patches of female and male lovely fairy-wrens. (a) Experiment 1: natural variability of each plumage patch (N = 23 females and 19 males). (b) Experiment 2: pre- and post-manipulation chromatic coordinates of the cheek patch (N = 35 females and 29 males). The x axis represents the relative stimulation of the S cone in relation to the VS cone; y represents relative stimulation of the M cone in relation to the VS and S cones; and z represents the relative stimulation of the L cone in relation to the VS, S and M cones.

Colour measurement

Plumage reflectance of captured birds was measured on five patches: head, cheek, shoulder, back and tail; we obtained five readings per patch from each bird. For experiment 2, we measured the reflectance of the cheek patch immediately before and after the colour manipulation. Reflectance measurements were taken in the bird-visible wavelength range (300–700 nm), using an Ocean Optics JAZ spectrometer and inbuilt PX-3 Pulsed Xenon light source, with a probe and a machined 45° angle end (UV-VIS fibre-optic reflectance, Ocean Optics, Largo, FL, U.S.A.). The probe end excluded all ambient light and maintained a fixed distance to the feather surface. Reflectance was calibrated relative to a white standard (Ocean Optics WS-2).

Reflectance spectra were down-sampled to 5 nm steps and summarized using psychophysical models of avian vision to describe chromatic variation (Vorobyev & Osorio, 1998; Vorobyev, Osorio, Bennett, Marshall, & Cuthill, 1998), as implemented by Delhey et al. (2015) using formulas in Cassey et al (2008)In birds, colour vision is mediated by four types of single cones that are sensitive to very short (VS), short (S), medium (M) and long (L) wavelengths. Variation in visual sensitivity between bird species is mainly in the VS and S cones and species can generally be ultraviolet sensitive (U-type) and violet sensitive (V-type). Since lovely fairy-wrens are U-type birds (Ödeen, Pruett-Jones, Driskell, Armenta, & Håstad, 2012), we used average U-type peak sensitivity: 372, 456, 544, 609 nm (Endler & Mielke, 2005). The noise-to-signal ratio of each cone type was calculated based on formula 10 in Vorobyev et al. (1998) from the average cone proportions of U-type birds from Hart (2001), (VS = 0.35 S = 0.6 M = 1 L = 1), combined with a Weber fraction of 0.1 for the L cone (Olsson, Lind, Kelber, & Simmons, 2017). As irradiance we used the spectrum of standard daylight (D65) (Vorobyev et al., 1998).

Visual models computed using the approach described above reduce each spectrum to a set of three (xyz) chromatic coordinates that define its position in avian visual space where the x axis represents the relative stimulation of the S cone in relation to the VS cone, the y axis represents relative stimulation of the M cone in relation to the VS and S cones and the z axis represents the relative stimulation of the L cone in relation to the VS, S and M cones. Chromatic distances are expressed in JNDs (just noticeable differences), whereby values below 1 are thought not to be discriminable (Kelber, Vorobyev, & Osorio, 2003). After carrying out the visual models, we averaged xyz coordinates of the five readings for each colour patch to have a single set of values per plumage patch (chromatic colour variation for each patch is shown in Fig. 2).

We summarized chromatic variation (xyz coordinates) with a principal component analysis (PCA) using a covariance matrix to maintain the JND units of the original data (Delhey et al., 2015). A separate PCA analysis was conducted for each colour patch, and in experiment 2 for the cheek patch before and after treatment (Appendix Table A4). All PCA analyses resulted in one factor ($PC1_{chroma}$) that explained more than 90% of chromatic variance, with low positive loadings for the x axis, intermediate negative values for y and high negative loadings for z (Appendix Table A4). Thus, spectra with high $PC1_{chroma}$ values provide low stimulation of the L cone relative to VS+S+M cones, and low stimulation of the M cone relative to S+VS. In other words, birds with high $PC1_{chroma}$ values had colours richer in shorter wavelengths (UV/blue) and poorer in longer wavelengths (red).

Within each sex, the colours of different (blue) patches were correlated (head, tail and back; Appendix Table A5); the back was excluded from further analysis because it was highly correlated with other blue patches (Appendix Table A5), and had the lowest visual impact compared to other plumage patches (hidden by wings and head). Cheek and shoulder (blue and red/brown, respectively) were less strongly correlated with one another and with other patches

(Table A5). Cheek patch was the most colourful patch in both sexes (Fig. 2, reflectance spectra in Appendix Fig. A2). Blue plumage patches were more colourful in males than females (more negative values indicate shortwave-rich colours) as was the red/brown shoulder patch (more positive values indicate longwave-rich colours) (Appendix Table A6, Fig. A2). Despite these differences, female and male homologous blue colour patches followed the same main axis of variation, which is different from the shoulder patch (Fig. 2).

Ethical note

Our study complied with the ASAB/ABS Guidelines for the Use of Animals in Research and was approved by the University of Melbourne animal ethics committee (register 1613868.1). Fieldwork was carried out under licence from Queensland Parks and Wildlife Service (WISP13237913), and birds were banded under Australian Bird and Bat Banding Scheme banding permits. All personnel involved were trained in the appropriate techniques to capture and handle birds. Birds were captured in mist-nets which were monitored continuously, and individuals were removed immediately upon capture. Birds were held for banding, measurements, and experiments for up to 30 min. Colour manipulation was applied in less than 2 min and was done with water-pen markers (instead of typical alcohol-based markers) with the purpose of being easily removable with water and self-maintenance behaviour. Individual appearance was not visibly changed after treatment and could only be confirmed by spectrophotometry. Birds were released back at their capture location, and were monitored weekly, through censuses and 1 h focal observations. No adverse effects on the birds' behaviour or survival were detected, as all birds were resighted on their territory for at least 1 month after the experiments, by which time the treatment is expected to have vanished completely (Delhey, Peters, Johnsen, & Kempenaers, 2007).

Statistical Analysis

Statistical analyses were performed with R v3.4.3 (The R Foundation for Statistical Computing, Vienna, Austria). We analysed behavioural responses to the mirror image stimulation using independent-samples Mann–Whitney tests to compare sex differences and paired-sample Wilcoxon tests to compare behavioural changes before and after the mirror was uncovered; we used the behaviours that changed (aggression, time spent close to and far from the mirror) as response variables in subsequent models. All these variables were correlated ($r_\rho > 0.25$, $P < 0.01$). To reduce the dimensionality of the behavioural response to the mirror, we ran a PCA based on the behavioural data collected after the mirror was exposed (Table 1). Variables aggression and time spent distant were log transformed to improve normality before running the PCA. We did a PCA separately for experiments 1 and 2 and a third PCA with both experiments (to facilitate comparing them; Table 1). Each PCA resulted in a single factor ($PC1_{aggression}$) that explained more than 50% of the variation, where positive values indicated higher aggressiveness and proximity to the mirror and less time spent far from it (Table 1). $PC1_{aggression}$ was used to compute the scores of behavioural responses to the mirror, hereafter ‘aggression score’.

For experiment 1, we used a general linear mixed model (GLMM) to assess which factors best explained variation in aggression score ($PC1_{aggression1}$) in the mirror test (assuming normal distribution of the error term for the dependent variable). As fixed effects in the model we included chromatic variation of the different colour patches ($PC1_{chroma1}$ for head, cheek, shoulder, tail), and other physical, social and ecological variables that might be related to aggressive behaviour: sex, body size, breeding stage (no nest, nesting, fledglings or unknown), number of neighbours (proxy for number of direct competitors in the area), Julian day (to account for differences within season) and time of day when tested. Group identity was included as a random effect to account for nonindependence of data collected from multiple birds of the same social group. Body size was estimated from the first factor of a PCA using tarsus, wing, and head–bill length, which explained 63% of variance, with high positive loadings for all variables (tarsus 0.5, wing 0.6, head–bill 0.7). We first report the results from the full model including all variables and complement this with multimodel inference using the Akaike information criterion corrected for small sample size (AICc) to select best fitting models based on AIC weights (Appendix Table A7). The AIC was calculated for models containing all possible combinations of fixed effects, using package ‘nlme’ (Pinheiro, Bates, DebRoy, & Sarkar, 2011) and the function ‘dredge’ from package ‘MuMIn’ (Barton, 2017).

From experiment 2, having found a relationship between cheek patch chromatic variation and aggression score (see Results), we tested whether aggression score ($PC1_{aggression2}$) was influenced by natural plumage (before manipulation) or by treatment type (‘blender’, ‘grey’, ‘blue’). In all models, we also included as fixed effects sex and its interaction with colour variable/treatment, and group identity as a random effect. We performed the same analysis excluding individuals that had colours outside of the natural range and that were detectable (see Appendix Tables A1 and A2 for ranges). We also tested for chromatic variation of the cheek patch post manipulation, and to control for individuals’ natural plumage colour we tested the interaction between their natural colour and the treatment applied (see Appendix, chromatic variation of the cheek patch post manipulation). To understand whether there were any differences between experiments, we tested whether aggression score or natural colour of the cheek varied between experiments 1 and 2, and we did not find any differences (see Appendix, differences between experiments).

In the main text, we report analyses from both females and males, testing for sex differences by using colour PCAs with the sexes combined, since colours follow the same axis of variation (Fig. 2). In Appendix Tables A8-A12, we report results using separate PCA colours for each sex and models for each sex that did not differ qualitatively from those reported in the main text.

In all the analyses, models did not include variables that were highly correlated (Pearson correlations, $r_\rho < 0.43$ for all variables included) to avoid problems of collinearity ($|r| > 0.7$; Dormann et al., 2013). GLMMs were performed using package lme4 (Bates & Maechler, 2010) and the significance of factors and degrees of freedom (Satterthwaite's method for approximating degrees of freedom) were assessed using the 'lmerTest' package (Kuznetsova, Brockhoff, & Christensen, 2017).

Table 1. Loadings for the principal component analysis (PCA) of behaviours (aggression, time spent close to the mirror, time spent far from the mirror) in the mirror image stimulation test, after uncovering the mirror

	Experiment 1	**Experiment 2**	**Experiments 1 and 2**
	$PC1_{aggression1}$	$PC1_{aggression2}$	$PC1_{aggression1+2}$
Aggression (pecks + swoops + displays)	0.51	0.56	0.55
Time spent close to the mirror	0.66	0.62	0.63
Time spent far from the mirror	-0.55	-0.54	-0.55
Eigenvalues (% of variance)	1.23 (50)	1.42 (67)	1.35 (61)

The PCA is for experiment 1, experiment 2 and both experiments combined.

Results

Behavioural responses to mirror image stimulation

Individuals changed their behaviour when the mirror was exposed, spending less time at intermediate distances and more time close to the mirror (experiments 1 and 2, paired-sample Wilcoxon test: $Z = -2.68$, $N = 106$, $P < 0.01$) as well as far from it ($Z = -3.09$, $N = 106$, $P < 0.01$). Two per cent of females and 17% of males performed displays towards the mirror (feathers erected, wings extended) and 40% of females and 60% of males pecked or swooped at the mirror (physical contact with the mirror; Appendix Fig. A3 and Supplementary Video S1). Females and males sang at higher rates before than after the mirror was exposed (mean songs/min ± SD: 2.12 ± 2.44 before, 1.42 ± 2.29 after; $Z = -2.84$, $N = 102$, $P < 0.01$). Both sexes sang at similar rates (songs/min) when the mirror was covered (independent-samples Mann–Whitney test: $Z = -1.04$, $P = 0.15$; female: 3.55 ± 2.48, $N = 58$; male: 2.41 ± 2.63, $N = 48$), but females sang at higher rates than males when the mirror was exposed ($Z = -2.81$, $P < 0.01$; female: 2.09 ± 2.69; male: 0.33 ± 0.59). Females that sang more were also more aggressive (correlation with female aggression score: $r_\rho = 0.32$, $P = 0.01$), but this was not the case in males (correlation with male aggression score: $r_\rho = -0.13$, $P = 0.38$).

Experiment 1: aggressive behaviour towards the mirror

Individuals with less blue cheek colour were significantly more aggressive towards their mirror image than those with bluer cheeks (Fig. 3). Other factors such as body size, breeding stage, density or season did not explain variability in aggressiveness in the full model (Table 2) and were also not present in the best fitted model (Appendix Table A7). The best fitting model explaining aggression score contained the fixed effects colour of the cheek (values for reduced model: $t_{35.60} = -2.76$, $P < 0.01$) and sex, with males being more aggressive towards the mirror than females (values for reduced model: $t_{38.99} = 3.58$, $P < 0.001$).

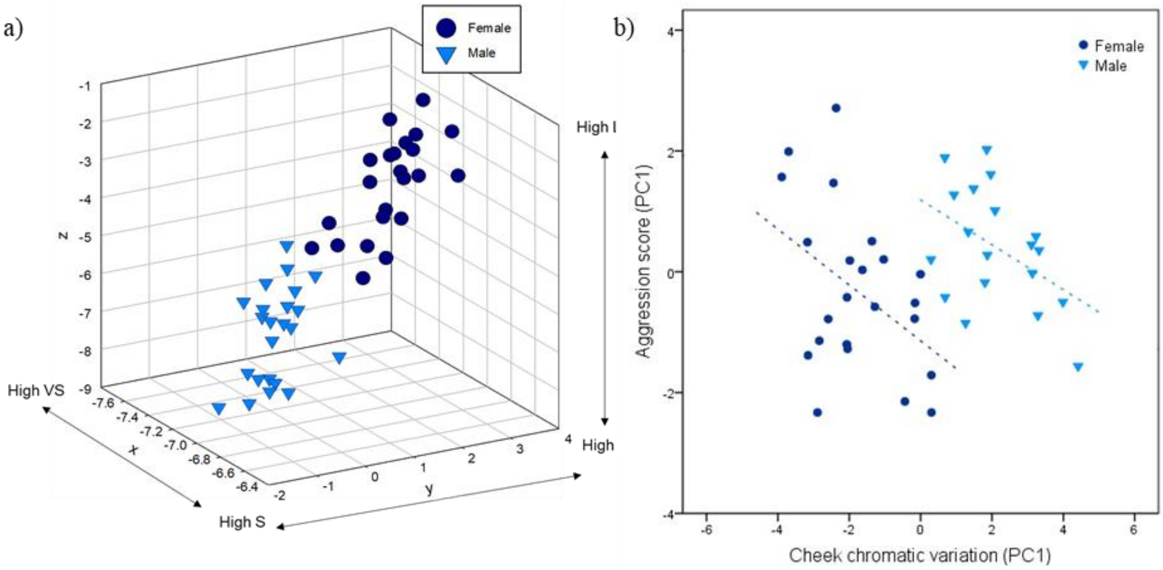

Figure 3. Experiment 1. (a) Chromatic variation of female and male cheek plumage colour represented within the tetrahedral colour space of birds. Most variation of the cheek colour is in the z axis, which represents long relative to shorter wavelength reflectance. Male and female cheek colours follow the same axis, although males have higher negative values towards UV and blue than females. Variation along the x axis represents stimulation of the VS cone relative to the S cone, the y axis represents stimulation of the M cone relative to the VS and S cones and the z axis represents stimulation of the L cone relative to the M, S and VS cones. Units of xyz are in JNDs (just noticeable differences). (b) Chromatic colour of the cheek patch ($\mathrm{PC1}_{\mathrm{chroma1}}$ of the cheek) in relation to aggression score ($\mathrm{PC1}_{\mathrm{aggression1}}$). Dashed lines depict the linear regression lines.

Table 2. The effect of predictor variables on aggression score, with group identity included as a random factor

Parameter	β	SE	*df*	*t*	*P*
Fixed effects					
Intercept	-30.91	72.15	26.35	-0.43	0.67
Sex[a]	1.78	1.16	2.77	1.53	0.14
Body size	-2.03e-02	0.20	21.64	-0.10	0.92
Cheek colour	**-0.43**	**0.19**	**28.99**	**-2.27**	**0.03**
Head colour	6.90e-02	0.11	27.79	0.65	0.52
Shoulder colour	-1.81e-02	2.62e-02	29.0	-0.69	0.49
Tail colour	-2.26e-02	5.06e-02	28.46	-0.45	0.66
Breeding stage[b]					
Nest	0.45	0.70	28.93	0.64	0.53
Fledglings	-0.60	0.95	28.39	-0.64	0.53
Unknown	0.48	0.64	26.41	0.75	0.46
No. of neighbours	1.54e-02	0.17	23.70	0.09	0.93
Julian day	1.92e-03	4.73e-03	26.29	0.41	0.69
Time of day	4.86e-04	7.94e-04	28.73	0.61	0.55
Random effect	σ^2				
Group identity	0.57				
Residual	0.83				

Significant predictors are in bold. Effects and associated statistics are based on the full model with all predictors.

[a] Sex is a categorical term and the reference is female.

[b] Breeding stage is a categorical term with four levels: no nest, unknown, nest and fledglings; the reference is no nest.

Experiment 2: colour perceived as an aggressive signal

Treatment type was related to aggression towards the mirror (Table 3), and males tended to be more aggressive than females although this difference was not significant (Table 3). Post hoc analysis revealed that individuals with colour expression increased by treatment 3 ‘blue’ (i.e. bluer colours) were significantly more aggressive than individuals from treatment 1 ‘blender’ (Fig. 4, Table 3); individuals in treatment 2 ‘grey’ group also tended to be more aggressive than treatment 1, although this difference was not significant (Table 3). Excluding individuals that had detectable post manipulation colours outside the natural range (see Appendix Tables A1 and A2 for ranges) gave results following the same trend (see Appendix, Results excluding individuals whose colour was outside the natural range). Results were also similar when we tested the effect of the continuous chromatic variation of the cheek patch post manipulation (see Appendix, Chromatic variation of the cheek patch post manipulation).

The subject's own colour (natural plumage colour before the manipulation) was not significantly related to aggression score ($t_{59.51}$ = 1.40, *P* = 0.16; Fig. 4), and neither was sex, either alone ($t_{56.70}$ = 0.29, *P* = 0.77) or in interaction with natural plumage (cheek PC1*sex: $t_{55.85}$ = -0.65, *P* = 0.52; Fig. 4a).

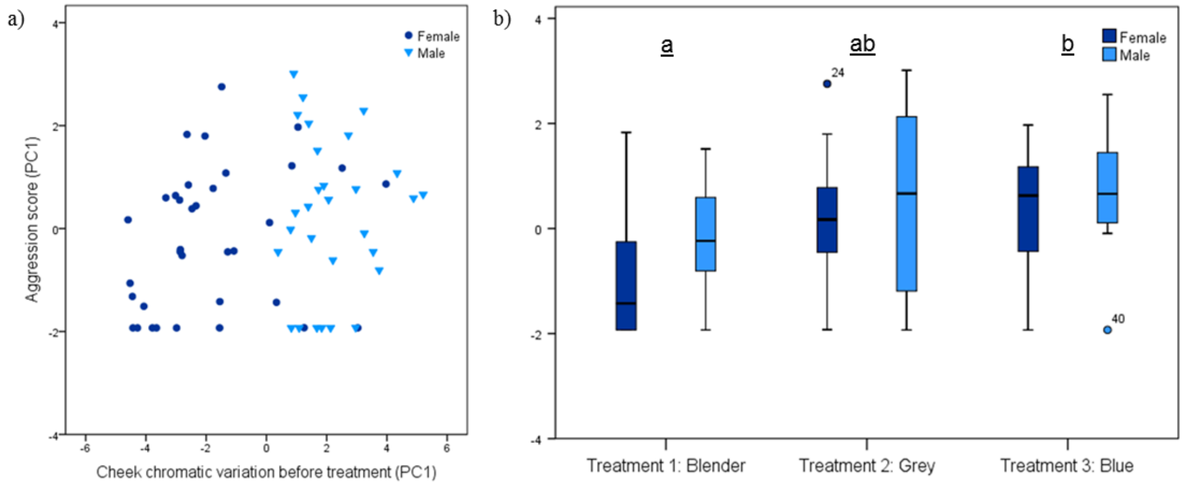

Figure 4. Experiment 2: relation between aggression score and (a) chromatic variation in cheek colour ($PC1_{chroma2}$) before treatment and (b) the three treatments (blender, grey, blue) for females and males. Box plots show the median and the interquartile range, whiskers indicate the values within 1.5 times the interquartile range and circles are outliers; different letters above bars indicate statistical significance (P<0.05).

Table 3. Effect of fixed predictors on aggression score ($\text{PC1}_{\text{aggression2}}$) in experiment 2 for treatments and post hoc comparisons between treatments

Parameter	*β*	SE	*df*	*t*	*P*
Intercept	**-1.23**	**0.39**	**56.03**	**-2.89**	**0.005**
Treatment[a]					
Blue	**1.30**	**0.58**	**55.52**	**2.26**	**0.027**
Grey	**1.44**	**0.53**	**50.29**	**2.71**	**0.009**
Sex[b]	1.09	0.54	33.99	2.02	0.051*
Treatment[a] * Sex[b]					
Blue * Sex	-0.57	0.85	50.02	-0.67	0.50
Grey * Sex	-1.16	0.76	39.66	-1.51	0.13
Treatment pairwise comparison					
Blue–grey	0.14	0.43	59.91	0.34	0.93
Blender–blue	**-1.07**	**0.43**	**59.62**	**-2.47**	**0.04**
Blender–grey	-0.92	0.40	60.00	-2.29	0.06*

Results are from the Tukey method for post hoc comparisons among the least-squares means treatments (treatment 1 = 'blender', treatment 2 = 'grey', treatment 3 = 'blue'; *N* = 70 comparisons), using package 'lsmeans' (Lenth, 2016). Significant predictors are in bold; an asterisk indicates marginal significance. Female *N* = 35, male *N* = 29.

[a] Treatment is a categorical term with three levels: 'blender', 'grey' and 'blue'; the reference is 'blender'.

[b] Sex is a categorical term and the reference is female.

Discussion

Using mirror image stimulation to experimentally assess aggressive behaviour in close-range same-sex interactions, we showed that female and male lovely fairy-wrens both reacted more aggressively to their mirror image reflection when they were less ornamented themselves (i.e. less blue or shortwave-rich colours). However, experimental manipulations of the cheek plumage revealed the opposite pattern: individuals with enhanced (richer in shortwaves) cheek colour behaved more aggressively towards the mirror image. Below we discuss these results and consider possible explanations for the difference between experiments.

Same-sex interactions with mirrored rivals

Males and females reacted strongly to the mirror image stimulation, either responding aggressively with both attacks and displays to the mirrored ‘rival’ or avoiding it. Aggressive responses to the mirror (Appendix Fig. A3 and Supplementary Video S1) were similar to behaviour observed in the field during natural competitive interactions, where both sexes are aggressive towards same-sex conspecifics (Leitão et al., 2019). Although some studies have shown that mirror tests do not elicit the same hormonal responses (Oliveira et al., 2005) or the same aggressive intensity as live opponents (Balzarini, Taborsky, Wanner, Koch, & Frommen, 2014; Elwood, Stoilova, McDonnell, Earley, & Arnott, 2014), the same studies show correlations between responses to mirror and real opponents. In contrast to natural aggressive interactions, the ‘rival’ does not operate independently of the subject (and perhaps differently between subjects), that is, the mirror does not initiate a behaviour, so the pace and (de-)escalation of contests are driven by the subject and mirrored by the rival.

In natural settings, long-range acoustic signals usually precede short-range visual signals in escalating interactions in birds (Bradbury & Vehrencamp, 1998). The mirror image stimulation test simulated a short-range interaction, where visual signals are expected to be most important. In fact, when individuals were exposed to the mirror, they reduced their song rate, consistent with the view that birdsong is a long-range signal. This allowed us to test for the effects of plumage colour, eliminating other confounding factors (such as song).

To our knowledge, our study is the first to explore the role of plumage coloration with the mirror image stimulation test. This method has promise for future studies (particularly to test short-range signals) and indeed could be broadly employed in studies of visual signals, much as playback experiments are widely used in acoustic signal studies (Searcy & Nowicki, 2000).

Plumage colour as a signal of aggression

In the first experiment, natural colour of the cheek patch in both females and males predicted behaviour in the mirror test. We found that individuals with less blue plumage colours were more aggressive towards the mirror than those with richer blue plumages. There are at least two possible interpretations of this result. First, individuals might have an intrinsic aggressive state which is tied to their plumage colour (McGraw & Hill, 2000a; Pryke & Griffith, 2006), with less colourful birds being more aggressive, independently of the opponent's colour. Alternatively, individuals are using colour as signal of social status (Griggio, Zanollo, & Hoi, 2010; Midamegbe et al., 2011), and be more likely to attack a less colourful 'rival' because it is perceived as weaker. The first explanation is unlikely, because if coloration was related to an intrinsic propensity for aggression, we should have observed a relationship between natural colour (before treatment) and aggression in experiment 2, which was not the case. Thus, we consider it more likely that colour is used as a signal in aggressive contexts.

Status signals have been shown to either increase or decrease aggression. For example, in red-winged blackbirds, *Agelaius phoeniceus*, male models with reddened epaulettes elicited greater responses from territorial owners and more displays from other males (other than the territory owner) compared to normal, dull or no epaulette models (Yasukawa et al. 2009). Also, in purple-crowned fairy-wrens, *Malurus coronatus*, 3D models in breeding colours received more aggression from resident breeder males than models in nonbreeding (dull) colours (Fan et al., 2018). In contrast, in house finches, *Carpodacus mexicanus*, duller males are dominant over bright males (McGraw & Hill, 2000a; McGraw & Hill, 2000b) and similar examples are found with acoustic signals, where individuals either respond strongly to low-threat signals or avoid high-threat signals (Cramer & Price, 2007; Hardouin, Reby, Bavoux, Burneleau, &

Bretagnolle, 2007; Searcy et al., 2008). It seems that status signals can either increase aggressive displays towards individuals that bear high-quality signals or deter attackers. In our experiment, we predicted that individuals would behave less aggressively to a more threatening signal because individuals were in a novel, unfamiliar environment (cage with mirror). We reasoned this to be more akin to a response to an intrusion onto the territory of a conspecific, rather than in defence of an individual's own territory, and in this context, individuals have more to lose if engaging in a fight due to owner–intruder asymmetry (Bradbury & Vehrencamp, 1998), where territorial ownership increases motivation to fight.

In the second experiment, manipulated colour of the cheek patch in both females and males predicted behaviour in the mirror test, but surprisingly, birds were more aggressive to increased than reduced coloration, in direct contrast to experiment 1. A few studies have shown similar results, where the experimental manipulation changed the expected result: in red bishops, *Euplectes orix,* less colourful individuals were more likely to win a contest, but when the colour was experimentally manipulated, more colourful individuals were more likely to win (Edler & Friedl, 2010); In northern cardinals, *Cardinalis cardinalis*, colour manipulation (either increasing or reducing) did not show any effect on dominance rank over food resources; however, naturally more colourful individuals were dominant over dull ones (Wolfenbarger, 1999a); In the crimson finch, *Neochmia phaeton*, natural carotenoid plumage coloration was positively related to the probability of winning a contest, but when the colour was manipulated, this pattern reversed, with the underlying natural colour negatively related to the probability of winning a contest, and no effect of the experimental colour on contest outcome (Young, Cain, Svedin, Backwell, & Pryke, 2016). We encourage other studies using mirror image stimulation to investigate visual signal function to assess responses to both unmanipulated and manipulated colour patches to test how widespread such contrasts are.

We considered several possible explanations for why birds reacted differently between experiments. First, our experimental manipulations of plumage colour may have resulted in ‘unnatural’ overall appearances of birds in the experimental treatments. If birds with the most altered cheek patches also deviated the most from ‘normal’ phenotypic appearance, this could have triggered higher levels of aggression. However, this was probably not the case, since most of the treated birds were within the natural variation, and even when excluding the individuals that were outside the natural range, the findings remained qualitatively similar.

Second, while plumage coloration may be an important signal in competition, other traits might be mediating aggression. Age, a factor that we could not control for, can affect aggressive behaviour (Edler & Friedl, 2010), as can previous experience (Collis & Borgia, 1992). Similarly, hormones such as testosterone can also mediate aggression and correlate positively with agonistic signals such as plumage (Tibbetts, 2014). In white-shouldered fairy-wrens, *Malurus alboscapulatus*, where females of different subspecies lack or possess male-like melanized black-and-white plumage (ornamentation), females of ornamented populations had higher levels of testosterone and were also more aggressive to a simulated territorial intrusion than those from populations with dull females, suggesting that testosterone may mediate aggression and female ornamentation (Enbody et al., 2018).

A third nonmutually exclusive possibility might be that plumage colour is a signal that has costs imposed by receivers through social punishment (Hurd & Enquist, 2005; Tibbetts, 2013; Tibbetts & Dale, 2004), and that increased aggression in response to enhanced colour plumage reflects punishment via social policing of a perceived signalling ‘cheat’. Some studies have shown that individuals with experimentally altered ornaments receive more aggression than ‘honest’ ones (Ligon & McGraw, 2016; Rohwer & Rohwer, 1978; Senar, 1999; Tibbetts &

Dale, 2004; reviewed in Webster, Ligon, & Leighton, 2018). In a controlled experiment, Tibbetts and Izzo (2010) showed that female paper wasps, *Polistes dominulus*, with their status signal altered and mismatched with behaviour resulted in social punishment, but when both were experimentally altered to match, individuals did not suffer more aggression. If receivers can detect false signals due to incongruence between different cues, aggression can be used as a punishment and maintain signals by social costs. In the present study, the correlation between the manipulated cheek colour and shoulder patch colour and body size became weaker after the manipulation (Appendix Table A3). This discrepancy between the manipulated signal, more blue cheek, and other phenotypic traits, such as body size, may have triggered a punishment response of the ‘rival’ with increased aggression, although further experiments designed specifically to test this are needed to understand the mechanisms regulating signal honesty.

Differences and similarities in females and males

Males were somewhat more aggressive to the mirror than females (significant in the reduced model in experiment 1, marginally nonsignificant in experiment 2), and males showed more aggressive displays towards the mirror, but in both sexes about half of the individuals interacted aggressively and physically towards it.

Although this species is dichromatic, both male and female cheek colour (which is the bluest and most reflective colour patch; Fig. 3, Appendix Fig. A2) was correlated with aggression across unmanipulated and manipulated colours (even if in opposite directions). It is likely that, although male and female colours are different, plumage is used to mediate social competition in both sexes. Indeed, the colours of both male and female cheek patches follow a similar pattern of variation in visual space (Fig. 3). Competitive signals are expected to evolve and benefit both sexes because of identical characteristics, ecological and social needs (sexual and non-sexual resources: West-Eberhard, 1979). In the tropics, females and males commonly have similar roles in territory defence (Stutchbury & Morton, 2001), and thus both sexes may benefit from competitive traits such as plumage coloration because of year-round territorial defence (Murphy et al., 2009a). This is likely to apply to lovely fairy-wrens, since they breed intermittently throughout the year, and maintain year-long territory defence. As adult survival in both females and males is high (Leitão et al., 2019), territory vacancies may be scarce and competitive signals might be effective during disputes.

Females and males sang at higher and similar rates before than after the mirror was exposed, suggesting that song might be used as a long-range signal that is supressed when individuals perceive a rival nearby. The function of song might differ between females and males, since we found that females sang at higher rates than males when the mirror was exposed, and females that sang more were also more aggressive. It is also possible that song has a similar signalling function, but males escalate more readily into physical aggression, whereas females spend more time signalling to reduce costs of fighting. Further controlled studies will be necessary to illuminate the function and sex differences in song in this species.

Conclusions

By analysing female and male lovely fairy-wren responses to natural and experimentally manipulated colour of their bright blue cheek patch, we showed that colour was related to aggressive behaviour, suggesting that plumage colour can function as a competitive signal in aggressive contexts and that this signal plays a role in intrasexual competition in both sexes.

In many tropical species both males and females are colourful (Dale et al., 2015) and it has been suggested that this might be linked to investment in territorial defence (Stutchbury & Morton, 2001). If selection favours more competitive individuals in these environments, then signals that mediate competition for resources may be important to reduce the probability of extreme consequences of frequent fighting (e.g. time, probability of injuries and death). However, the fact that correlational and experimental results showed opposite patterns indicates that more research is needed to establish the precise nature of the link between plumage colour and aggression. Particularly, future experiments should find a treatment that effectively reduces the colour to understand whether dull birds are still more aggressive (as in experiment 1). The results of this study do not rule out that plumage colour is also used in intersexual interactions such as mate choice, since birds breed year-round unlike most Australian fairy-wrens (reviewed in Leitão et al., 2019), and future work should address how plumage traits relate to reproductive and nonreproductive resources (e.g. territories), to infer other functions and selective pressures.

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Appendix

Experiment 2: Treatment effect

Descriptors of treatments are given in Table A1. Treatments mostly varied in the z axis and most or half of the individuals fall within the natural variation (Table A1).

To determine how close each manipulated colour was to the natural range, we analysed the differences between each treated colour and the nearest pre-manipulated one in colour space given by Euclidean distance and expressed in JND units (just noticeable differences), with values below 1 JND thought not to be discriminable (Kelber, Vorobyev, & Osorio, 2003). In both females and males, the Euclidean distances average for ‘blender’ and ‘grey’ were close to or below 1 JND, meaning that painted colours resembled natural colours, but the ‘blue’ treatment average was higher than 2 JND, meaning that this treatment may be perceived as different from the natural colour (Table A2). Overall, treatment 1 ‘blender’ maintained coloration, treatment 2 ‘grey’ increased coloration within the natural range and treatment 3 ‘blue’ increased coloration within and outside the natural range (Tables A1-A2; see Methods).

Chromatic variation of the cheek patch post manipulation

From experiment 2, having found a relationship between cheek patch chromatic variation and aggression score (see Results), we tested whether aggression score ($PC1_{aggression2}$) was influenced by chromatic variation of the cheek patch post manipulation and included as fixed effects sex and its interaction with colour as a dependent variable and group identity as a random effect. To understand whether response to the mirror test was influenced by the interaction between the individual's natural colour and the treatment applied, we ran another model with the same terms but using as fixed factors the PCA of the chromatic difference between before and after treatment colour (see Table A4 for PCA loadings).

Results showed that chromatic variation of the cheek patch post manipulation was positively correlated with aggression score (Table A14, Fig. A4), suggesting that enhanced plumage (colour of 'rival' in mirror) elicited a more aggressive response from test subjects. Males and females did not differ in their overall aggressive response, and the interaction between colour and sex was marginally significant (Table A14, Fig. A4).

Colour treatment controlling for individual colour (difference between natural colour and manipulated colour) showed again that individuals with increased plumage reflection were more aggressive towards their enhanced mirror reflection; sex and its interaction with colour difference was marginally related to aggression (Table A14, Fig. A4).

Differences between Experiments

To analyse differences between experiment type, we built two GLMMs to test whether aggression (PCA aggression computed on both experiments: $\text{PC1}_{\text{aggression1+2}}$; Table 1), or natural colour of the cheek patch (PCA chroma computed on both experiments: $\text{PC1}_{\text{chroma1+2}}$; Table A4), varied between experiments 1 and 2. We included bird and group identity as random factors to account for nonindependence in the data.

Aggression score did not vary between experiments ($t_{13.91}$ = -0.62, *P* = 0.55), nor did the natural colour of the cheek patch ($t_{3.11}$ = 1.25, *P* = 0.29).

Results excluding individuals whose colour was outside the natural range

If we exclude the individuals that are outside the natural range (*N* = 9 excluded, five from female 'blue' treatment, three from male 'blue' treatment and one from 'grey' treatment) results are similar to those reported in the main text (Table 3): treatment type was related to aggression towards the mirror and males tended to be more aggressive than females (Table A13). Individuals with colour expression increased in treatment 2 'grey' were more aggressive than those in treatment 1 'blender'; individuals in treatment 'blue' were more aggressive than individuals from treatment 1 'blender' although because of the smaller sample size this difference was marginally nonsignificant (Table A13).

Figures

Figure A1. Male and female lovely fairy-wrens are dichromatic. Blue colours are a result of coherent light scattering of feather barbs (structural colours; Prum, 2006), and the red and blacks are a result of melanin pigment deposition, identified based on the shape of reflectance spectra described in Delhey (2015). Photo credits Ana V. Leitão.

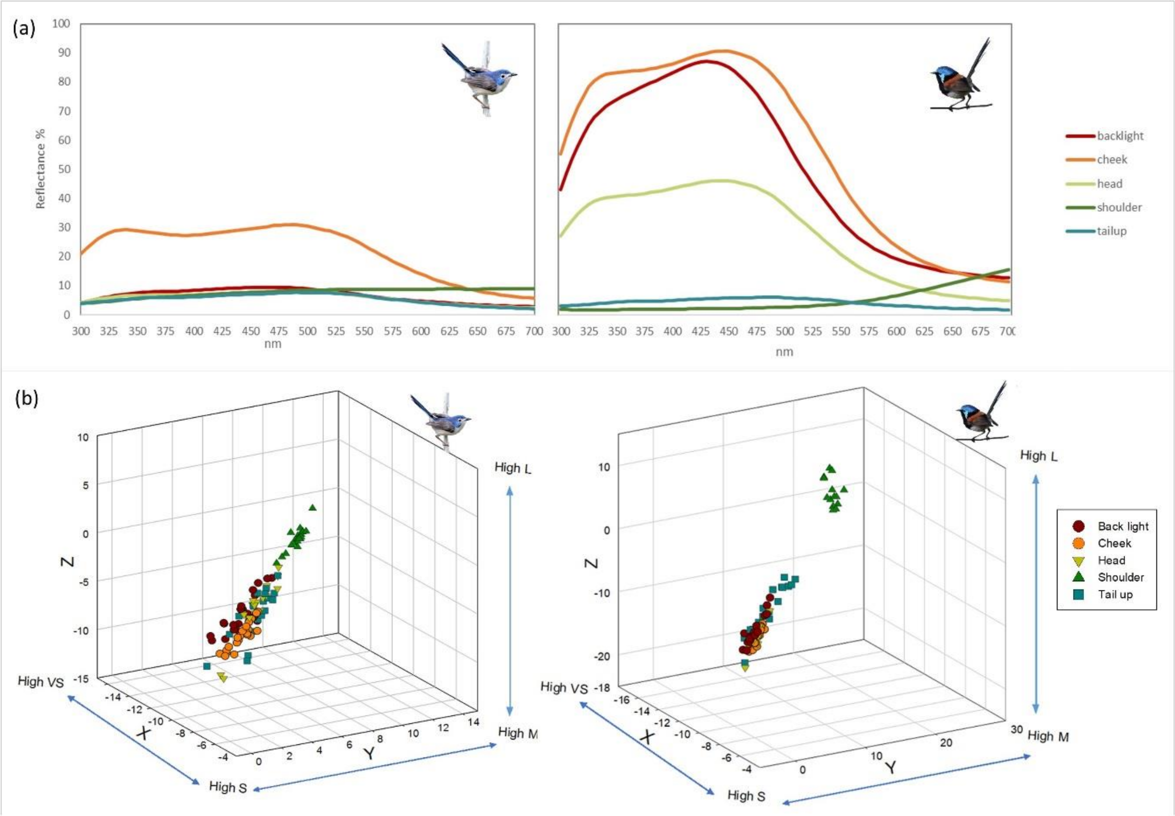

Figure A2. Natural variability of each plumage patch in female and male lovely fairy-wrens (N = 23 females and 19 males). (a) The average reflectance spectra for each colour patch and for each sex. (b) Graphical representation of chromatic coordinates (xyz) that define their position in the avian visual space. Here, x represents the relative stimulation of the S cone in relation to the VS cone, y represents relative stimulation of the M cone in relation to the VS and S cones and z represents the relative stimulation of the L cone in relation to the VS, S and M cones. Sample from experiment 1.

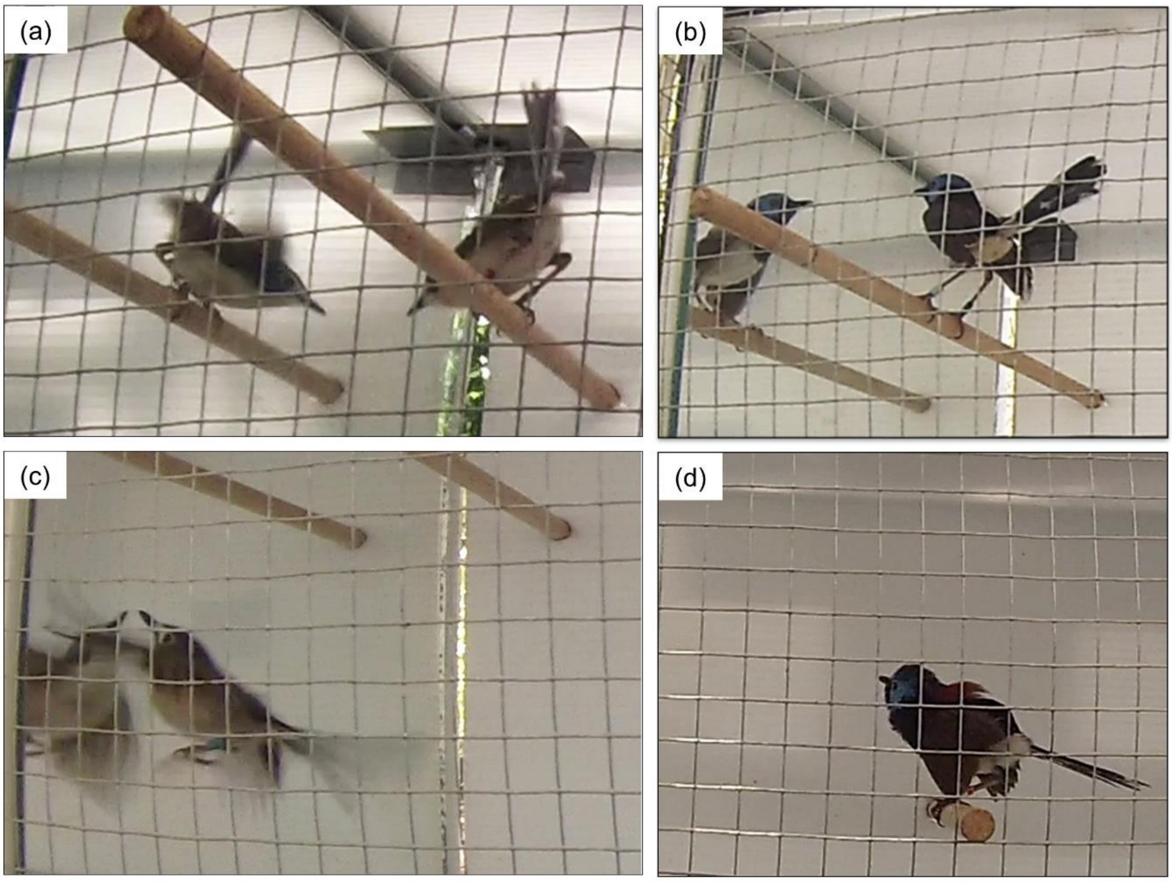

Figure A3. Female and male aggressive displays towards the mirror ‘rival’: (a) female pecking the mirror, (b) male displaying (wings extended) and pecking, (c) female swooping at the mirror and (d) male displaying towards the mirror (feathers erected, wings extended). Photo credits Ana V. Leitão.

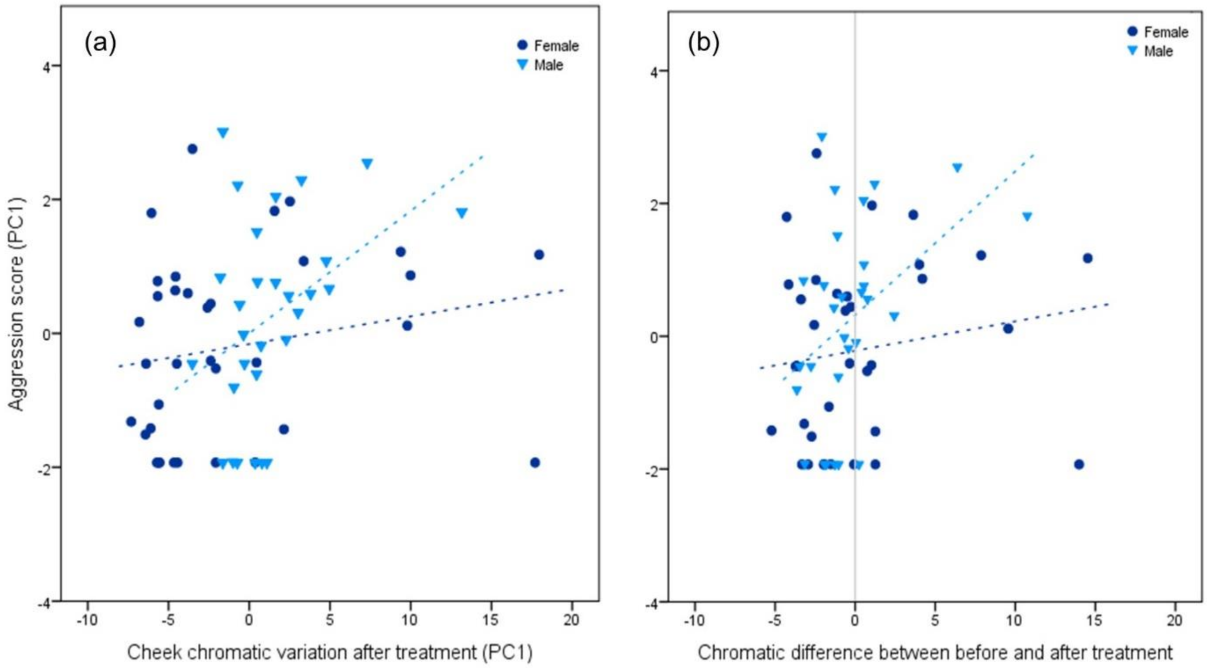

Figure A4. Relation between aggression score and chromatic variation in experiment 2: (a) chromatic variation in cheek colour (PCAxyz) post-treatment; (b) within-individual colour changes between natural colour and treatment. Grey dashed vertical line at 0 indicates no difference in colour (between natural and treatment), negative values indicate decreased colour and positive values indicate increased colour. In both plots, the x axis scale is represented in JNDs (just noticeable differences). Dashed lines depict the linear regression lines.

Tables

Table A1. Descriptors of visual coordinates for experiment 2

		Female				Male			
		Mean	Minimum	Maximum	Within natural range (%)	Mean	Minimum	Maximum	Within natural range (%)
Premanipulation (*N* = 35 F, 29 M)	x	-6.993	-7.57	-6.605		-6.479	-6.917	-5.981	
	y	1.697	-0.21	3.253		-1.995	-3.599	-0.482	
	z	-5.561	-12.94	-2.703		-7.559	-10.31	-6.139	
Blender (*N* = 12 F, 10 M)	x	-6.985	-7.497	-6.615	100	-6.738	-7.129	-6.456	90
	y	2.821	1.025	4.252	83	-1.379	-3.125	0.727	90
	z	-5.392	-11.513	-2.302	92	-7.939	-11.91	-5.452	80
Grey (*N* = 13 F, 11 M)	x	-7.118	-7.437	-6.649	100	-6.805	-7.137	-7.137	73
	y	2.607	0.884	5.162	69	-1.754	-2.783	-2.783	100
	z	-5.317	-9.592	-2.888	100	-9.199	-13.22	-13.219	82
Blue (*N* = 10 F, 8 M)	x	-7.692	-8.387	-7.241	60	-7.137	-8.488	-6.721	50
	y	-0.11	-2.899	1.882	60	-2.783	-5.914	-2.195	75
	z	-15.86	-27.613	-6.801	50	-13.22	-21.45	-9.931	63

Table A2. Detectability of differences in colour treatment

	Female			Male		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Pre-manipulation – blender	0.893	0.254	1.495	0.729	0.104	1.746
Pre-manipulation – grey	0.765	0.296	1.962	0.988	0.208	2.955
Pre-manipulation – blue	5.334	0.665	14.766	2.859	0.281	11.208

Differences in colour space between each manipulated colour and the nearest pre-manipulated colour, given by Euclidean distance and expressed in JND units (just noticeable differences), with values below 1 JND thought not to be discriminable (Kelber, Vorobyev, & Osorio, 2003).

Table A3. Female and male matrix correlations of PCA xyz chromatic coordinates of the cheek before and after the manipulation for experiment 2

		All		Female		Male	
		Correlation *(r)*	*P*	Correlation *(r)*	*P*	Correlation *(r)*	*P*
Back	Cheek before	0.27	0.03	0.69	< 0.001	0.14	0.49
	Cheek after	0.61	< 0.001	0.75	< 0.001	0.05	0.80
Head	Cheek before	0.56	< 0.001	0.71	< 0.001	0.21	0.28
	Cheek after	0.65	< 0.001	0.77	< 0.001	0.17	0.39
Shoulder	Cheek before	-0.29	0.02	0.51	0.001	0.02	0.92
	Cheek after	0.04	0.7	0.58	< 0.001	0.02	0.92
Tail	Cheek before	0.39	0.001	0.64	< 0.001	0.09	0.65
	Cheek after	0.28	0.02	0.72	< 0.001	0.02	0.92
Body size	Cheek before	0.53	< 0.001	0.16	0.34	0.18	0.36
	Cheek after	0.26	0.03	0.18	0.30	0.19	0.35

Female *N* = 35, male *N* = 29.

Table A4. Loadings for principal component analysis (PC1$_{\text{chroma}}$) of xyz colour for experiments 1 and 2 and both experiments combined, with sexes together (used in the main analysis)

	Variables	**Experiment 1 PC1$_{\text{chroma1}}$**	**Experiment 2 PC1$_{\text{chroma2}}$**	**Experiment 1 + 2 PC1$_{\text{chroma1+2}}$**
Cheek	x	0.112	0.063	0.073
	y	-0.708	-0.684	-0.711
	z	-0.697	-0.727	-0.699
	Variance (%)	83	83	85
Back	x	0.099		-0.067
	y	-0.771		-0.093
	z	-0.629		-0.993
	Variance (%)	92		92
Head	x	0.0524		-0.0519
	y	-0.605		-0.173
	z	-0.795		-0.983
	Variance (%)	90		92
Tail	x	-0.234		-0.047
	y	-0.032		-0.018
	z	-0.972		-0.999
	Variance (%)	92		99
Shoulder	x	0.607		0.441
	y	-0.603		-0.462
	z	-0.518		-0.769
	Variance (%)	92		58
Cheek postmanipulation	x		-0.046	
	y		-0.313	
	z		-0.949	
	Variance (%)		89	
Cheek difference	x		-0.065	
	y		-0.232	
	z		-0.971	
	Variance (%)		95	

Cheek difference: difference between before and after treatment. Variable x represents the relative stimulation of the S cone in relation to the VS cone; y represents relative stimulation of the M cone in relation to the VS and S cones; and z axis represents the relative stimulation of the L cone in relation to the VS, S and M cones. For all blue colours (cheek, back, head, tail), negative values explained variation in y and z; spectra with high PC1chroma values provide low stimulation of L cone relative to VS+S+M cones, and M relative to S+VS, i.e. high PC1 values had colours richer in shorter wavelengths (UV/blue) and poorer in longer wavelengths (red). For red/brown colours (shoulder), positive values explain variation in x, and negative values explain variation in y. Thus, low PC1 values represent high stimulation of M relative to S+VS.

Table A5. Correlation between colour patches (PCA xyz chromatic coordinates) in females and males

		Female		Male	
		Correlation *(r)*	*P*	Correlation *(r)*	*P*
Cheek	Back	0.61	< 0.0001	0.31	0.03
	Head	0.62	< 0.0001	0.37	0.01
	Tail	0.53	< 0.0001	0.34	0.01
	Shoulder	0.46	< 0.001	0.11	0.46
Back	Head	0.96	< 0.0001	0.52	< 0.0001
	Tail	0.91	< 0.0001	0.48	< 0.0001
	Shoulder	0.74	< 0.0001	0.03	0.81
Head	Tail	0.88	< 0.0001	0.89	< 0.0001
	Shoulder	0.69	< 0.0001	0.09	0.55
Tail	Shoulder	0.49	< 0.0001	0.22	0.14

Female N = 58, male N = 48.

Table A6. Comparison of female and male plumage homologous colour patches

	Pillai	*F*	*df*	*P*
Cheek	0.85	196.22	3,10	< 0.0001
Back	0.82	160.12	3,10	< 0.0001
Head	0.88	239.56	3,10	< 0.0001
Tail	0.14	5.3375	3,10	< 0.001
Shoulder	0.86	205.66	3,10	< 0.0001

The table shows the results of a multivariate analysis of chromatic coordinates (xyz) for each colour patch.

Table A7. Predictors of aggressive behaviour of females and males in response to the mirror image stimulation test

Model order	Predictors of aggression	Intercept	Loglik	AIC$_c$	ΔAICc	ὠ$_i$
1	Cheek colour + Sex	1.23	-63.06	137.78	0	0.38
2	Sex	0.36	-65.48	140.04	2.26	0.12
3	Body size + Cheek colour + Sex	0.87	-60.57	141.51	3.73	0.06
4	Density + Cheek colour + Sex	-0.17	-62.11	141.51	3.73	0.06
5	Breeding Stage + Cheek colour + Sex	1.21	-63.89	142.18	4.39	0.04

Summary of model selection showing the main predictors of aggression score, using the Akaike information criterion corrected for small sample size (AICc) in package ‘MuMIn’ (Barton, 2017). Loglik: restricted log-likelihood; ΔAICc: difference between the best model and the given model; ὠi: Akaike weight of a given model. Group identity was also included as a random factor. The models with the lowest AICc and ΔAICc < 2 were retained and considered to be the best supported (Burnham & Anderson, 2002).

Table A8. Loading for principal component analysis (PC1chroma) of xyz colour for experiments 1 and 2 and both experiments for each sex

		Experiment 1		**Experiment 2**		**Experiment 1 + 2**	
		$PC1_{chroma1}$		**$PC1_{chroma2}$**		**$PC1_{chroma1+2}$**	
	Variables	F	M	F	M	F	M
Cheek	x	-0.005	0.109	-0.038	0.048	-0.038	0.058
	y	-0.485	-0.534	-0.288	-0.562	-0.305	0.529
	z	-0.874	-0.838	-0.956	-0.826	-0.951	0.847
	Variance (%)	87	88	95	78	92	81
Back	x	-0.109	0.063			-0.063	-0.022
	y	-0.263	-0.583			-0.102	0.479
	z	-0.959	-0.810			-0.993	0.877
	Variance (%)	90	91			99	89
Head	x	-0.108	-0.031			-0.120	-0.026
	y	-0.263	-0.353			-0.072	-0.114
	z	-0.959	-0.935			-0.990	-0.993
	Variance (%)	93	91			98	99
Tail	x	-0.209	-0.266			-0.098	-0.045
	y	-0.039	-0.012			-0.043	-0.016
	z	-0.977	-0.964			-0.994	-0.998
	Variance (%)	96	87			99	99
Shoulder	x	0.674	0.752			-0.310	0.397
	y	-0.649	-0.565			0.034	0.284
	z	-0.352	-0.338			0.949	-0.872
	Variance (%)	63	99			96	54
Cheek postmanipulation	x			-0.052	-0.073		
	y			-0.226	-0.317		
	z			-0.973	-0.946		
	Variance (%)			98	92		
Cheek difference	x			-0.061	-0.085		
	y			-0.197	-0.360		
	z			-0.978	-0.928		
	Variance (%)			97	93		

F: female; M: male. Cheek difference: difference between before and after treatment. Variable x represents the relative stimulation of the S cone in relation to the VS cone; y represents relative stimulation of the M cone in relation to the VS and S cones; and z represents the relative stimulation of the L cone in relation to the VS, S and M cones. For all blue colours (cheek, back, head, tail), high PC1 values had colours richer in shorter wavelengths (UV/blue) and poorer in longer wavelengths (red). For red/brown colours (shoulder), low PC1 values represent high stimulation of M relative to S+VS.

Table A9. Results of principal component analysis for body size (aggression, time spent close to the mirror, time spent far from the mirror), for each sex

	Female	Male
Tarsus length	0.40	0.52
Wing length	0.65	0.62
Head-bill length	0.64	0.59
Eigenvalues (% of variance)	1.16 (45)	1.21 (49)

Table A10. Results of principal component analysis (PCA) of activity variables (aggression, time spent close to the mirror, time spent far from the mirror) in the mirror image stimulation test, after uncovering the mirror for experiments 1 and 2, and both experiments combined, for each sex

	Experiment 1 $PC1_{aggression1}$		Experiment 2 $PC1_{aggression2}$		Experiment 1 and 2 $PC1_{aggression1+2}$	
	Female	Male	Female	Male	Female	Male
Aggression (pecks + swoops + displays)	0.56	0.49	0.60	0.52	0.58	0.48
Time spent close to the mirror	0.58	0.78	0.62	0.63	0.61	0.67
Time spent far from the mirror	-0.60	-0.39	-0.50	- 0.59	-0.54	-0.56
Eigenvalues (% of variance)	62	41	70	65	65	55

Table A11. Experiment 1: summary of linear model showing all parameters of aggression score ($\text{PC1}_{\text{aggression1}}$), for each sex

	Female				Male			
Parameter	*β*	SE	*t*	*P*	*β*	SE	*t*	*P*
Fixed effects								
Intercept	-155	158	-0.98	0.35	7.91	162.24	0.05	0.96
Body size	-1.97e-02	0.33	-0.06	0.95	-0.14	0.42	-0.33	0.75
Cheek colour	-0.59	0.40	-1.47	0.17	-0.35	0.46	-0.76	0.47
Head colour	0.10	0.24	0.42	0.68	0.04	0.29	0.15	0.89
Shoulder colour	0.44	0.46	0.95	0.36	0.011	0.06	0.18	0.86
Tail colour	-2.11e-02	0.14	-0.15	0.88	4.28e-03	0.12	0.04	0.97
Breeding stage[a]								
Nest	-0.65	1.93	-0.33	0.74	0.18	1.66	0.11	0.92
Fledglings	-2.32	2.20	-1.06	0.31	-0.39	2.27	-0.17	0.87
Unknown	-0.29	1.31	-0.22	0.83	0.53	1.89	0.28	0.79
No. of neighbours	-5.81e-02	0.28	-0.21	0.84	0.25	0.43	0.58	0.58
Julian day	1.01e-02	1.04e-02	0.98	0.35	-6.73e-04	0.01	-0.06	0.95
Time of day	4.16e-04	1.49e-03	0.28	0.78	1.68e-03	2.18e-03	0.77	0.47

Female *N* = 22, male *N* = 19. Global model: Aggression score ~ Cheek colour + Head colour + Shoulder colour + Tail colour + Body size + Breeding stage + Nr. Neighbours + Julian day + Time of day.

[a] Breeding stage is a categorical term with four levels: no nest, unknown, nest and fledglings; the reference is no nest.

Table A12. Experiment 2: effect of fixed predictors on aggression score (PC1aggression2), models built for each sex

	Female					Male				
Parameter	*β*	SE	*df*	*t*	*P*	*β*	SE	*df*	*t*	*P*
Natural colour										
Intercept	-0.89	0.25	1,33	0	1.0	7.18e-11	0.26	1,27	0	1.0
Cheek colour	0.14	0.10	1,33	1.39	0.18	0.04	0.21	1,27	0.19	0.84
Treatment										
Intercept	-0.82	0.39	2,32	-2.05	**0.04**	-0.44	0.45	2,26	-0.98	0.34
Treatment[a]										
Blue	1.29	0.59	2,32	2.19	**0.03**	0.78	0.68	2,26	1.15	0.26
Grey	1.21	0.55	2,32	2.18	**0.03**	0.60	0.63	2,26	0.96	0.34
Colour after treatment										
Intercept	-8.32e-11	0.25	1,33	0	1.0	5.18e-11	0.24	1,27	0	1.0
Cheek colour	0.04	0.03	1,33	1.16	0.25	0.16	0.07	1,27	2.15	**0.04**
Colour difference										
Intercept	-8.70e-11	0.24	1,33	0	1.0	5.55e-11	0.24	1,27	0	1.0
Cheek colour	0.04	0.05	1,33	0.90	0.37	0.19	0.08	1.27	2.30	**0.02**

Female *N* = 35, male *N* = 29.

[a] Treatment is a categorical term with three levels: ‘blender’, ‘grey’ and ‘blue’; the reference is ‘blender’.

Table A13. Effect of fixed predictors on aggression score (PC1aggression2) in experiment 2 for treatment and comparison between treatments, excluding individuals that were outside the natural range and had detectable differences in colour (see Table A1 and A2)

Parameter	*β*	SE	*df*	*t*	*P*
Intercept	-1.10	0.39	48.86	-2.76	**0.007**
Treatment[a]					
Blue	1.30	0.73	48.98	1.77	0.08 *
Grey	1.39	0.54	45.48	2.55	**0.01**
Sex[b]	1.08	0.55	30.17	1.96	0.059 *
Treatment[a] * Sex[b]					
Blue * Sex	-1.23	1.00	36.13	-1.22	0.22
Grey * Sex	-1.13	0.79	32.84	-1.43	0.16

Significant predictors are in bold; an asterisk indicates marginal significance. Female *N* = 30, male *N* = 25. Models were performed using packages lme4 (Bates & Maechler, 2010) and the significance of factors and degrees of freedom were assessed using the ‘lmerTest’ package (Kuznetsova, Brockhoff, & Christensen, 2017).

[a] Treatment is a categorical term with three levels: ‘blender’, ‘grey’ and ‘blue’; the reference is ‘blender’.

[b] Sex is a categorical term; the reference is female.

Table A14. Effect of fixed predictors (colour postmanipulation and the difference between natural colour and resultant colour) on aggression score (PC1$_{\text{aggression2}}$) in experiment 2 for both sexes

	Colour after treatment					Colour difference				
Parameter	*β*	SE	*df*	*t*	*P*	*β*	SE	*df*	*t*	*P*
Intercept	0.01	0.27	59.66	0.04	0.97	0.34	0.25	59.93	1.34	0.19
Cheek colour	0.19	0.08	54.96	2.37	**0.02**	0.23	0.09	52.29	2.63	**0.01**
Sex[a]	-0.18	0.34	37.78	-0.52	0.61	-0.56	0.31	34.89	-1.78	0.08*
Cheek colour * Sex[a]	-1.15	0.09	56.26	-1.71	0.09*	-0.19	0.09	52.81	-1.89	0.06*

Significant predictors are in bold; an asterisk indicate marginal significance. Female *N* = 35, male *N* = 29. Models were performed using packages lme4 (Bates & Maechler, 2010) and the significance of factors and degrees of freedom (Satterthwaite’s method for approximating degrees of freedom) were assessed using the ‘lmerTest’ package (Kuznetsova et al., 2017).

[a] Negative coefficients indicate that females are less aggressive.

Chapter 4

Pairing, paternity and reproductive success in relation to plumage colouration in Lovely fairy-wrens

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Abstract

Sexual selection has promoted the evolution of complex ornamental traits, which in many organisms convey information about individual quality. We assessed whether plumage ornamentation in male and female lovely fairy-wrens *Malurus amabilis* is sexually selected. We analysed the correlation between plumage colour and measures of quality or reproductive success to understand if plumage colour might act as honest signal. We also examined the relationship between plumage traits and patterns of pairing and paternity. We found evidence of assortative mating based on blue plumage colours and parental care, but female and male plumage colour were uncorrelated with measures of individual quality. Microsatellite analyses of paternity indicate that the lovely fairy-wren has extremely high levels of extra-pair paternity, with 53% of offspring resulting from extra-pair mating and 58% of broods containing offspring sired by males other than the social mate. Lower rates of extra-pair paternity occurred where density of breeding pairs was lower. Female and male plumage colour did not predict the proportion of extra-pair offspring in their own nest, but less colourful males obtained higher extra-pair and total paternity. These results suggest that males and females have different strategies in relation to plumage colour and mating behaviour and emphasises the importance of looking at both sexes in studies of sexual selection. The current findings together with previous study, suggest that plumage colour functions in intra and inter-sexual competition.

Keywords: Assortative mating, Extra-pair paternity, Female ornaments, Mate choice, Mating strategy, Plumage colouration, Promiscuity, Sexual selection

Introduction

In males of many species, sexual selection has resulted in elaborated traits that do not serve to enhance survival, but instead function in competition or to attract females (Darwin, 1871) by advertising male phenotypic or genetic quality (Andersson, 1994). There is increasing interest in whether similar or different selective forces drive such traits in females, with comparative studies (Dale et al., 2015; Price & Eaton, 2014; Soma & Garamszegi, 2015), as well as empirical studies (Brunton & Li, 2006; Illes & Yunes-Jimenez, 2009; Jacobs, Fair, & Zuk, 2015; Kraaijeveld et al., 2004; Odom et al., 2016; Price, Yunes-Jiménez, Osorio-Beristain, Omland, & Murphy, 2008; Tobias et al., 2011; Topp & Mennill, 2008) conducting parallel analyses on both sexes, though the mechanisms that have shaped ornamental traits in females remain debated (Clutton-Brock, 2009; Tobias et al., 2012).

The expression of elaborate traits in females is most likely the result of direct selection, rather than just a by-product of selection on males (reviewed in Amundsen, 2000a; Amundsen & Pärn, 2006; LeBas, 2006). Direct selection can explain female functional signals, either to increase reproductive success (sexual selection), or to increase access to limited resources via competition (social selection; Heinsohn et al., 2005; LeBas, 2006; West-Eberhard, 1983). There is now abundant evidence across many species to support Darwin's (1871) hypothesis that males that express the most conspicuous ornaments obtain more, or higher quality matings. Here we explore the possibility that plumage colouration in both sexes of one species is involved in mate choice, and/or competition for increased chances of reproductive success.

There is theoretical and empirical support for a process of 'mutual mate choice' when both sexes are ornamented (Heinig et al., 2014; Hunt et al., 1999; Kraaijeveld et al., 2004; MacDougall & Montgomerie, 2003). Morphological traits, such as plumage colours, may be used to reliably assess potential mates of both sexes if they are correlated with aspects of individual quality. Males, like females, may benefit from choosing mates based on direct benefits such as phenotypic quality (parental care, body condition) or on genetic quality (age, parasite resistance and immune capacity), which could impact their reproductive success (Amundsen & Pärn, 2006). Traits may also serve as status signals, and evolve through competition over mates or other resources (Tobias et al., 2012). Mate choice and social competition are not mutually exclusive: one trait may signal to attract mates and simultaneously be used in competitive contexts (Berglund, Bisazza, & Pilastro, 1996; Griggio, Serra, Licheri, Monti, & Pilastro, 2007; Tobias et al., 2011). Both social competition and mate choice can lead to assortative patterns of pairing and mating because individuals with similar traits are likely to pair or mate more frequently than expected by chance (Burley, 1983).

Many socially monogamous bird species engage in extra-pair mating (Griffith, Owens, & Thuman, 2002). In species that form pair bonds, but sometimes mate with extra-pair individuals, mate choice involves both choosing a social partner and choosing the genetic parent(s) for the offspring (Moller, 1992). Where pair bonds are lifelong, individuals may have limited opportunity to choose a social mate, due to limited availability of mates or vacant territories (loss of territory, risk of predation, etc.). Such constraints on mate choice in the context of social pair formation may increase an individual’s willingness to engage in extra-pair mating behaviour. On the one hand, individuals may not gain much in being selective about extra-pair partners. On the other hand, if opportunities are limited or benefits are high, individuals might show a preference for high quality extra-pair mates. Either way, extra-pair behaviour may be driven by male and/or female mate choice, though most studies have focused on males only (Forstmeier, Nakagawa, Griffith, & Kempenaers, 2014; Griffith et al., 2002).

The objective of this study was to test the hypothesis that plumage colour is a sexual signal in socially monogamous female and male lovely fairy-wrens (*Malurus amabilis*). We tested whether: (i) plumage colour was related to indicators of individual quality, such as body condition and parental care; (ii) individuals paired assortatively based on elaborate secondary sexual traits; (iii) females chose extra-pair mates on the basis of plumage colour; and (iv) individuals with the most elaborate plumage had the highest reproductive success. We did this by examining the relationship between plumage colour, pairing patterns, and extra-pair paternity (EPP).

The lovely fairy-wren is a dichromatic species, with both male and female exhibiting conspicuous, but qualitatively different forms of colouration. Both sexes have mostly blue plumage colours that are a result of coherent light scattering of feather barbs (structural colours; Prum, 2006). Colouration signals aggression in the context of same-sex competition (Leitão et al., *in press*), but its role in mate choice is unknown. In closely-related fairy-wren species, the timing of moult into nuptial plumage in males is a sexual signal and a predictor of extra-pair mating success (Brouwer et al., 2011; Cockburn et al., 2008; Dunn & Cockburn, 1999; Karubian, 2002). However, in lovely fairy-wrens, males retain their bright plumage year-round after reaching maturity, and do not undergo a seasonal moult (Leitão et al., 2019) so other traits, such as plumage colour, are likely to be implicated as sexual signals.

Fairy-wrens (*Malurus*) are cooperative breeders and socially monogamous, yet exhibit extremely high but variable rates of EPP, ranging across species from 4.4% to 76% of offspring (Cockburn, Brouwer, Double, Margraf, & van de Pol, 2013; Kingma et al., 2009; Mulder et al., 1994; Rowe & Pruett-Jones, 2013). A recent comparative study in this family showed that multiple factors predicted the variation of EPP, including the number of helpers, number of neighbours and high male density, and inbreeding avoidance (Brouwer et al., 2017). This study and a previous review suggest that there is no single explanation for variation in EPP (Griffith et al., 2002), so here we considered several possible predictors, while testing whether individual variation in EPP is related ornament expression.

We predicted that if plumage colour is sexually selected: a) plumage colour is an indicator of individual quality that can serve as a cue in mate choice; b) female and male lovely fairy-wrens display positive assortative social pairing based on plumage colour, where they would possibly achieve higher reproductive success; and c) plumage colouration is used in extra-pair mate choice.

Methods

Study system and general field methods

Lovely fairy-wrens are a non-migratory species endemic to the wet tropics of North Queensland, Australia. Field work was conducted in northern Cairns area, Far North Queensland (16.87˚S, 145.75˚E), where a colour-banded population was established in 2013 and monitored annually until 2017 (for further details see (Leitão et al., 2019).

Lovely fairy-wrens occupy territories and breed virtually all year, but with a peak in activity in the Austral Spring, between August and November (Leitão et al., 2019). Progeny may remain on their natal territory as helpers and assist in subsequent broods (Leitão et al., 2019). Delayed dispersal is exclusive to males, and females were never seen to help (Leitão et al., 2019). In our study population, 34.7% of 49 groups included male helpers.

Unbanded individuals were captured by targeted mist-netting and individually marked with a numbered metal band (Australian Bird and Bat Banding Scheme) and a unique combination of colour bands. At capture we measured plumage colour (see below) and collected a small blood sample (less than 50 µl) from the brachial vein which was stored in 100% ethanol for later DNA extraction. To assess body size and condition, we weighed individuals (± 0.5 g) and measured tarsus length (± 0.05 mm).

Once individuals were released, we monitored their territories and group members, nesting behaviour and neighbouring territories (density of breeding pairs) weekly. We estimated annual adult survival (presence or absence) by the recovery rate of banded adult breeders (for details see Leitão et al., 2019).

Nesting and parental care

Lovely fairy-wrens exhibit bi-parental care, with the major contribution of the male being in post-hatch offspring care (Leitão et al., 2019). Females build the nests and incubate, while males remain near the nest and often engage in courtship feeding. Females lay 2 to 3 eggs, and can re-nest several times a year after unsuccessful and successful attempts (Leitão et al., 2019). We searched for and monitored nests, colour-banding 7 – 9 day-old nestlings and taking a small blood sample for paternity analysis. Putative parents were determined by extensive observations of each pair or group, including their attendance at the nest.

Both males and females defend the nest and provision the nestlings and fledglings (Leitão et al., 2019). Parental care was quantified by recording nestling provisioning as the number of visits by the female, male and helpers from video recordings (GoPro HD Hero2) of active nests. Adults normally resumed feeding within a maximum of 10 min of placing the camera and could be distinguished by their colour bands. We recorded nests for about one hour in the morning (between 0620-1230) when nestlings were day 2-5 days (early stage, n=10) and 7-9 days (late stage, n = 9). As much as possible, we recorded food provisioning two times per nest (n=6), but some nests do not have repeated measures due to predation of later stage nestlings (n=4), or because the breeding attempt was found at later stages (n=3).

Microsatellite analysis, Paternity Assignment and genetic similarity

Genomic DNA was extracted from blood samples using a standard salt extraction method (Bruford et al., 1992). In total, 209 individuals were genetically screened by the Australian Genome Research Facility (Melbourne, Australia) for up to 25 loci (Appendix: Table A1). From these, we selected 14 loci for analysis (Table 1) that had high variability, high call rates, did not deviate significantly from Hardy–Weinberg equilibrium, and had low null allele frequencies (Fnull < 0.03, except for Smm1, Mala14, and Mcymu8 where Fnull = 0.073-0.118; this was taken into account during paternity assignment). For each locus, we calculated the probability of maternal and paternal exclusion, based on allele frequencies of 117 breeders. In total across all 14 loci, we had 173 alleles (X = 12.357) and the combined probability of incorrectly assigning a random male as the sire was less than 1% (Table 1).

To assign parentage of each offspring (*N* = 58 nestlings) we assumed that the female that built, incubated and fed the nestlings was the genetic mother (in this species, groups have only one adult female). This assumption was valid because no female mismatched the offspring at more than two loci. The social male was accepted as the sire if the nestling mismatched the male’s genotype at 2 or fewer loci. Additionally, to assign potential extra-pair sires, we used CERVUS 3.0.7. (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton,

1998) to select the most likely male sire from the population, by using the log likelihood score (LOD) for each male in the population and comparing to the offspring (pair LOD score). Each paternity assignment was then checked, and in most cases, we accepted the assignment, unless the selected male had two or more mismatches and evidence suggested that the assigned was not the most likely sire, for e.g., sibling from the same brood or future brood, or a male from a distant population (more than 5 km away, maximum natal and breeder dispersal observed was 1.2 km (Leitão et al., 2019)). In one case only, we accepted the second assigned male, and in six cases we did not accept the assigned male and did not assign paternity, since there were no other males that matched.

We also examined parentage in groups with fledglings or subordinates (N = 19), to determine if they were likely to be offspring of the breeding pair. Individuals were only included in the analysis if they matched the putative mother at 2 or fewer loci. Rates for EPP for nestlings and fledglings/subordinates were analysed separately, but results are similar (Table 2). In total, we were able to assign paternity for 70 out of 77 nestlings and fledglings/subordinates.

We estimated relatedness within social pairs to assess genetic similarity between females and their social and extra-pair mates, by calculating the pairwise r coefficient following the method of Queller and Goodnight (1989) and using the software Coancestry (v.1.0.1.9; Wang, 2011). Pairs were categorised as incestuous (full sibling pairing and parent-offspring) when pairwise relatedness was within the range of the mean ± 1.5 * SD of known first-order relatives (Brouwer et al., 2017) (N = 17, X = 0.46, range: 0.30 - 0.70) .

Table 1. Description of microsatellite loci used for parentage analyses, based on 117 dominant, putatively unrelated birds.

Locus	N individuals typed	Nr Alleles	Heterozygosity		Maternal exclusion probability	Paternal exclusion probability	Null allele frequency
			Expected	Observed			
Mcymu7 [1]	116	4	0.573	0.543	0.163	0.282	0.023
Mcymu8 [1]	111	22	0.917	0.721	0.701	0.824	0.118
Tmm6 [2]	111	58	0.971	0.946	0.875	0.933	0.011
Smm1 [3]	83	7	0.621	0.518	0.225	0.404	0.079
MaLa2 [4]	115	17	0.874	0.861	0.588	0.742	0.004
MaLa4 [4]	115	5	0.637	0.652	0.216	0.361	-0.016
MaLa5 [4]	114	6	0.63	0.658	0.207	0.354	-0.021
MaLa6 [4]	115	5	0.671	0.67	0.242	0.404	-0.002
MaLa7 [4]	115	5	0.482	0.513	0.122	0.274	-0.033
MaLa8 [4]	115	8	0.699	0.73	0.277	0.444	-0.025
MaLa10 [4]	115	13	0.827	0.809	0.486	0.658	0.007
MaLa13 [4]	115	8	0.772	0.809	0.39	0.571	-0.030
MaLa14 [4]	115	9	0.808	0.704	0.452	0.629	0.073
MaLa18 [4]	114	6	0.763	0.763	0.369	0.549	-0.003
Combined	117	173			0.99	0.99	0.023

Maternal exclusion probability is the probability that at a given locus, a random parent will not match the nestling when no parent is known. Paternal exclusion probability is the probability that a candidate father will be excluded assuming that the mother is known. References for microsatellite are: [1] (Double, Dawson, Burke, & Cockburn, 1997); [2] (Adcock & Mulder, 2002); [3] (Maguire, Guay, & Mulder, 2006); [4] (Thrasher, Butcher, Campagna, Webster, & Lovette, 2017).

Reproductive and paternity success

We calculated ‘reproductive success’ of each pair as the number of fledglings per year that survived for more than a month. We also calculated male ‘within-pair paternity’ success as the number of offspring a male sired in his own territory; male ‘extra-pair paternity’ success as the total number of extra-pair offspring he sired in the nests of other males, and ‘total paternity’ success as the sum of a male’s within and extra-pair paternity success. These were annual measures for each territory (we did not have more than one nest of the same individual within a year).

Measures of colouration and analysis

Spectral reflectance properties of female and male plumage were measured at capture using an Ocean Optics JAZ spectrometer and inbuilt PX-3 Pulsed Xenon light source, with a probe and a machined 45° angle end (UV-VIS fiber-optic reflectance). Before measuring each bird, the spectrophotometer was calibrated relative to a white standard (Ocean Optics WS-2) and a dark reference. Methods are described in Leitao *et al.* (*in press*). Briefly, we took five readings of different body parts, and summarised reflectance spectra to describe chromatic variation using psychophysical models of avian vision (Vorobyev & Osorio, 1998; Vorobyev et al., 1998), as implemented by Delhey *et al.* (2015) using formulas from Cassey *et al.* (2008). Colour vision in birds is mediated by four types of single cones that are sensitive to very short (VS), short (S), medium (M) and long (L) wavelengths. Visual models reduced each spectrum to a set of three (xyz) chromatic coordinates that define its position in avian visual space, where distances are expressed in JNDs (just noticeable differences). Axis x represents the relative stimulation of the S cone in relation to the VS cone, y axis represents relative stimulation of the M cone in relation to the VS and S cones, and axis the z represents the relative stimulation of the L cone in relation to the VS, S and M cones.

Within each sex, the colours of different (blue) patches are moderately correlated, and cheek and head were the most colourful patches measured and with higher visual impact in each sex (Leitão et al., *in press*), so we used these two patches in this analysis. For each sex, we summarised chromatic variation (xyz coordinates) with a Principal Component Analysis (PCA) using a covariance matrix to maintain the JND units of the original data (Delhey et al., 2015). PCA analysis resulted in one factor (PC1) that explained between 73 - 99% of chromatic variance, with high PC1 values representing low stimulation of the L and M cone relative to the VS+S cones, so high PC1 values indicated individuals with colours richer in shorter wavelengths (UV/blue, Table 2). We ran PCAs separately for each sex, rather than combining the sexes (cf. appendix and main text analyses in Leitão et al., *in press*), because females and males are dichromatic, and we sought to test for patterns of assortative mating between sexes without combining them in a PCA.

In a subsample of re-captures, we found that colour was moderately repeatable between years (Appendix Table A2), so we pooled colour data from different years.

Table 2. Loadings for principal component analysis ($PC1_{chroma}$) of xyz colour for cheek and head for each sex separately. Variable x represents the relative stimulation of S cone in relation to VS cone; y represents relative stimulation of M cone in relation to VS and S cones; and z axis represents the relative stimulation of L cone in relation to VS, S and M cones

	Variables	**$PC1_{chroma}$**	
		Female	Male
Cheek	x	-0.05	0.12
	y	-0.25	-0.68
	z	-0.96	-0.71
	Variance	91 %	73 %
Head	x	-0.13	-0.04
	y	-0.01	-0.09
	z	-0.99	-0.99
	Variance	99 %	95 %

Statistical analysis

All statistics were performed in R (version 3.4.4) and Rstudio (version 1.1.414).

To assess whether plumage traits are indicators of individual quality or related with survival costs, we related plumage colours to body condition, estimated by the scaled mass index following (Peig & Green, 2009), body size, parental care, and survival, using general linear models. To test whether body size and body condition were correlated with plumage traits, we built a linear model with normal error distribution for females and males separately and included cheek and head colour as predictors. For the parental care analysis, we built a general linear mixed model (GLMM) with Poisson distribution and male and female number of feeding visits separately as response variables, controlling for effects of brood size and brood age (Leitão et al., 2019) and partner's provisioning rate (since female and male provisioning rate were correlated, see Results) and including the duration of the observation as an offset to control for differences in the duration of the observations, and nest ID as a random effect to account for repeated measures of the same nest. To determine whether there was an association between plumage colour and individual survival, we used a general linear mixed model with survival as a binomial response variable (survived more than 12 months after capture/colour measurement = 1; died before one year = 0), cheek colour, head colour and its interaction with sex as predictors, and with year as a random effect, to account for annual differences in survival probability.

We analysed patterns of assortative pairing and mating, by testing correlations between females and their social partner and extra-pair mates in terms of colour (cheek and head plumage), body size (tarsus length) and body condition. We also tested if there was a correlation between females and their social partners for parental care.

To determine whether plumage colouration predicts female and male breeding success (number of fledglings per year), we used a GLMM with Poisson distribution, to test the predictors plumage colour of both female and male (cheek and head colour) and their interaction, presence of helpers, and territory size (as a measure of territory quality). We included female ID and male ID as random factors to account for repeated measures of the same individuals in different years.

We analysed the proportion of extra-pair offspring to understand possible patterns of female choice by testing the influence of female's own plumage colour, her partner's colour, interaction between female and partner's colour, number of neighbouring territories (measure of density and opportunities), presence of helpers, and territory size on proportion of nestlings in brood that were EP offspring. We fitted the model with binomial distribution and included female ID and male ID as random factors.

To determine which factors influence male paternity success, we used three GLMMs with normal error distribution and log transformed response variables for the total number of offspring that a male sired: 'within-pair paternity', 'extra-pair paternity' and 'total paternity' (within+extra-pair). We assessed the effects on paternity of the male's own plumage colour, his female partner's colour and the interaction between the two, number of neighbouring territories, presence of helpers, and territory size. We included male ID and partner's ID as a random factor. Because of the low number of incestuous pairs and general low relatedness between individuals (see Results), we did not include relatedness in the analysis.

In the models of breeding success, female mate choice and male paternity success, we present reduced models, due to the higher number of predictors tested, where non-significant terms ($P > 0.2$) were sequentially removed from the models by stepwise backward procedures, starting with interactions terms. We also present full models for comparison.

To test whether females prefer more colourful or better condition males, we compared extra-pair sires with the within-pair male, using a Wilcoxon paired-test, for plumage colour (head and cheek), body size and body condition.

GLMs were performed using packages lme4 (Bates & Maechler, 2010) and the significance of factors and degrees of freedom (Satterthwaite's method for approximating degrees of freedom) were assessed using the lmerTest package (Kuznetsova et al., 2017). Differences in sample sizes between tests are due to incomplete data on reflectance spectra, physical measurements, or parental care for some individuals.

Results

Individual quality, survival and plumage colour

We found no relationship between plumage colour and body condition or body size in females and males (Appendix Table A3). Females provided more parental care to bigger broods and when their partner provisioned more (Table 3). Male provisioning was not related with any of the predictors tested (Table 3). Furthermore, no association between survival and plumage colour or its interaction with sex was found (Appendix Table A4).

Table 3. Parental care (number of visits to the nest) of females and males. Significant predictors in bold, marginal to significance italicised.

	Female (observations N = 16)				Male (observations N = 16)			
	β	SE	*z*	*P*	*β*	SE	*z*	*P*
Intercept	-9.00	0.60	-14.79	< **0.0001**	-7.24	0.56	-12.94	**<0.0001**
Cheek colour	0.008	0.09	0.09	0.92	-0.12	0.18	-0.68	0.49
Head colour	-0.04	0.04	-1.07	0.28	-0.06	0.06	-1.07	0.28
Partner’s prov.	0.14	0.05	2.62	**<0.01**	0.03	0.03	1.00	0.31
Brood day	0.08	0.07	1.18	0.23	0.01	0.07	0.16	0.86
Brood size	0.39	0.16	2.39	**0.01**	0.07	0.17	0.45	0.65
Random Effect	σ^2				σ^2			
Nest id	0.00 (*N* = 11)				0.00 (*N* = 11)			

Assortative pairing

Females with more colourful blue head and cheek patches paired with males who also had bluer cheeks (female cheek and male cheek: *r* = 0.32, *N* = 36, *P* = 0.05; female head and male cheek: *r* = 0.46, *N* = 36, *P* = 0.004, Figure 1 (a) and (b)). No relation was found between other colour patches (female cheek and male head: *r* = -0.26, *N* = 36, *P* = 0.11; female head and male head: *r* = 0.07, *N* = 36, *P* = 0.66). Females and males also paired assortatively with respect to parental care (*r* = 0.70, *N* = 21, *P* <0.001, Figure 1 (c)). No patterns of assortative pairing were found based on body size (tarsus length *r* = 0.02, *N* = 37, *P* = 0.91), or body condition (*r* = -0.15, *N* = 37 *P* = 0.37).

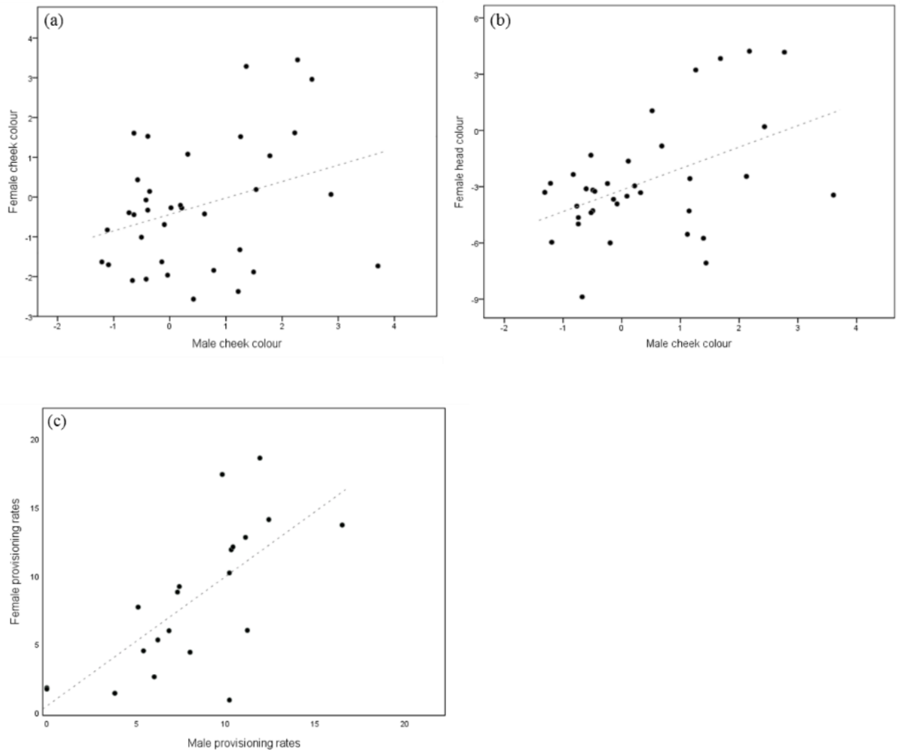

Figure 1. Assortative pairing based on plumage colour of the female cheek and male cheek (a), female head and male cheek (b), and provisioning rates of females and males (c). Dashed line shows regression line.

Frequency of extra-pair paternity

Paternity analysis showed that 53% of 58 offspring resulted from extra-pair mating and 58% of 29 broods analysed contained offspring sired by male other than the female's social partner (Table 4). Of 19 juveniles from 12 groups analysed, 32% were extra-pair. Most broods were fathered by a single male (72% of 29 broods: 12 by the social pair male only and 9 by a single extra-pair male). The remaining broods were fathered by up to three different males: 5 broods were fathered by the social male and one extra-pair male; 3 broods were fathered by multiple extra-pair males.

Table 4. Frequency of extra-pair nestlings and juveniles.

	Nestlings	**Juveniles**	**Total**
% EPP Nestlings (N)	53.44 (31)	31.57 (6)	48.05 (37)
% Broods with EPP	58.62 (17)	33.33 (4)	51.21 (21)
Total offspring sampled	58	19	77
Total broods	29	12	41
Nr incomplete Broods	6	2	8

Reproductive and paternity success

Ten percent of pairs were incestuous pairings (3 of 33 pairs where both male and female were genotyped). Five out of 7 nestlings in all 3 broods from these pairs were EP offspring.

Reproductive success was unrelated to female or male colour or any other variable tested (Table 5), though in the full model more colourful females tended to have higher reproductive success (Female head colour, Table 5). Females tended to have more extra-pair offspring when they had helpers in their group (Table 6), and the proportion of nestlings in the brood that were EP offspring did not depend on the female's or her mate's colour or any other variable tested (Table 6).

Table 5. Annual reproductive success of females and males (number of fledglings produced that survived). Significant predictors in bold, marginal to significance italicised. *N* = 26.

Full Model	β	SE	z	P
Intercept	0.19	0.67	0.28	0.77
Female cheek colour	0.16	0.18	0.85	0.39
Female head colour	0.33	0.17	1.89	*0.057*
Male cheek colour	-0.13	0.14	-0.93	0.34
Male head colour	-0.13	0.14	-0.93	0.34
Presence of helpers[a]	0.35	0.53	0.66	0.50
Territory size	0.19	0.13	1.42	0.15
Female cheek * Male cheek	0.27	0.20	1.31	0.18
Female head * Male cheek	0.15	0.19	0.80	0.42
Random Effect	σ^2			
Female ID (*N* = 16)	0.00			
Male ID (*N* = 19)	0.00			
Reduced Model	β	SE	z	P
Intercept	0.70	0.35	1.97	**0.04**
Female head colour	0.16	0.11	1.39	0.16
Random Effect	σ^2			
Female ID	0.00			
Male ID	0.00			

[a] Helper is categorical term and reference is negative (no) presence of helpers
Reduced model: Backward step-wise procedures were used to remove non-significant variables (P > 0.2).

Table 6. Proportion of nestlings in brood that were EP offspring. Significant predictors in bold, marginal to significance italicised. *N* = 28.

Full Model	β	SE	z	P
Intercept	-13.33	10.69	-1.24	0.21
Female cheek colour	0.25	1.02	0.24	0.80
Female head colour	-2.29	2.12	-1.07	0.28
Male cheek colour	-9.21	8.39	-1.09	0.27
Male head colour	1.35	1.25	1.08	0.28
Neighbours	3.97	2.66	1.49	0.13
Presence of helpers[a]	0.85	2.39	0.35	0.72
Territory size	-0.58	0.80	-0.71	0.47
Female cheek * Male cheek	-0.92	1.12	-0.82	0.40
Female head * Male cheek	-3.66	2.87	-1.27	0.20
Random Effect	σ^2			
Female ID (*N* = 18)	2.59-e10			
Male ID (*N* = 21)	10			
Reduced Model	β	SE	z	P
Intercept	-4.71	2.98	-1.57	0.11
Neighbours	0.98	0.76	1.28	0.20
Presence of helpers[a]	3.22	1.90	1.69	*0.09*
Random Effect	σ^2			
Female ID	0.0			
Male ID	6.8			

[a] Presence of helpers is categorical term and reference is negative (no) presence of helpers
Reduced model: Backward step-wise procedures were used to remove non-significant variables (P > 0.2).

The probability of a male gaining within-pair paternity was negatively related with number of neighbouring territories, with males having higher rates of within-pair paternity in less dense areas (Table 7, Figure 2 (a)). Less colourful males tended to have higher ‘within-pair paternity success when paired with less colourful females (reduced model: Male’s colour * Partner’s colour, Table 7). Males had higher extra-pair paternity when they were in more dense areas, when they had less colourful cheeks, and when assorted negatively with their partner (negative relation between male’s colour and female’s colour, Table 7). Male total paternity success was again negatively related with cheek colour (Table 7, Figure 2 (b)).

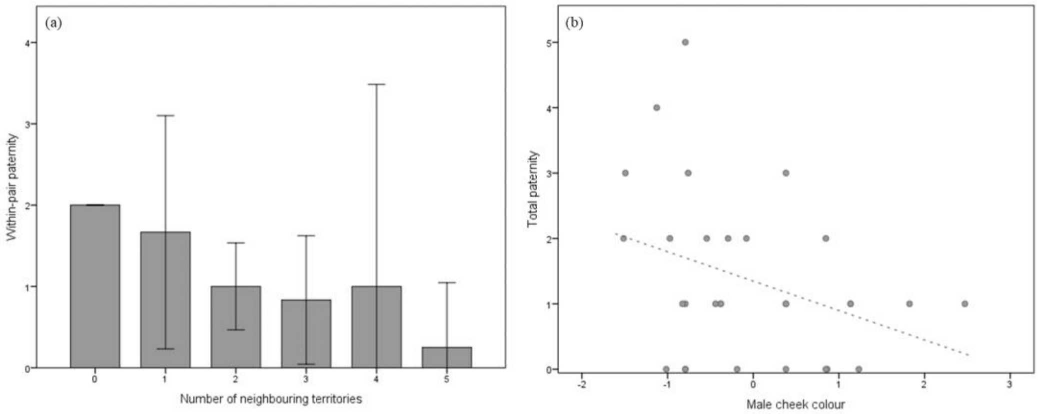

Figure 2. Relationship between number of neighbours and total number of within paternity (a), and male cheek colour and total paternity (b). Dashed line shows regression line.

Table 7. General linear mixed model showing the predictors of within-, extra-pair and total paternity success of males. Significant predictors in bold, marginal to significance italicised.

	Within-pair paternity (*N* = 28 male-years)				Extra-pair paternity (*N* = 30)				Total = Within and extra-pair paternity (*N* = 28)			
Full Model	β	SE	*t*	*P*	β	SE	*t*	*P*	β	SE	*t*	*P*
Intercept	0.26	0.07	3.49	**0.004**	0.11	0.07	1.49	0.15	0.36	0.09	3.90	**0.003**
Male cheek colour	-0.02	0.05	-0.54	0.59	-0.06	0.05	-1.32	0.20	-0.07	0.06	-1.23	0.24
Male head colour	0.005	0.02	0.16	0.86	-0.02	0.02	-0.83	0.41	-0.01	0.03	-0.56	0.58
Partner’s cheek colour	0.005	0.03	0.13	0.89	0.04	0.03	1.20	0.26	0.04	0.04	0.91	0.40
Partner’s head colour	0.008	0.01	0.79	0.43	0.001	0.01	0.17	0.86	0.009	0.01	0.73	0.47
Neighbours	-0.19	0.09	-2.00	*0.08*	0.14	0.09	1.51	0.16	-0.05	0.11	-0.53	0.61
Presence of helpers [a]	-0.03	0.11	-0.31	0.75	-0.03	0.11	-0.31	0.75	-0.06	0.13	-0.45	0.65
Territory size	0.003	0.08	0.04	0.96	0.06	0.08	0.78	0.44	0.03	0.09	0.32	0.74
Male cheek * Partner’s cheek	0.03	0.03	0.78	0.44	-0.06	0.03	-1.78	*0.09*	-0.03	0.04	-0.73	0.47
Male cheek * Partner’s head	0.01	0.01	1.41	0.17	-0.001	0.01	-0.14	0.88	0.01	0.01	1.06	0.30
Random effect	σ^2				σ^2				σ^2			
Male ID	0.07e-14 (21)				1.50e-17 (22)				0.0 (21)			
Female ID	0.009 (18)				0.009 (18)				0.007 (18)			
Residual	0.02				0.02				0.04			
Reduced Model												
Intercept	0.24	0.04	5.75	**<0.001**	0.14	0.03	4.09	**0.004**	0.33	0.03	8.70	**<0.0001**
Male cheek colour	-0.02	0.04	-0.53	0.60	-0.07	0.03	-2.71	**0.04**	-0.09	0.03	-2.38	**0.02**
Partner’s cheek colour					0.04	0.02	1.80	0.12				
Partner’s head colour	0.008	0.008	0.95	0.35								
Neighbours	-0.21	0.08	-2.65	**0.02**	0.16	0.07	2.25	**0.04**				
Male cheek * Partner’s cheek					-0.07	0.02	-2.54	**0.02**				
Male cheek * Partner’s head	0.02	0.01	2.07	*0.051*								
Random effect	σ^2				σ^2				σ^2			
Male ID	0.0 (21)				0.0 (22)				0.0 (21)			
Female ID	0.006 (18)				0.001 (18)				0.0 (18)			
Residual	0.02				0.02				0.04			

[a] Presence of helpers is categorical term and reference is positive (yes) presence of helpers

Reduced model: Backward step-wise procedures were used to remove non-significant variables (P > 0.2).

Extra-pair male traits

Females and their extra-pair males mated assortatively for some colour patches (female cheek – male head: *r* = 0.80, *N* = 12, *P* = 0.001); no other pattern was found in relation to colour (female head – male head: *r* = -0.16, *N* = 12, *P* = 0.60; female cheek – male cheek: *r* = -0.01, *N* = 12, *P* = 0.96). No patterns of assortative pairing were found based on body condition (*r* =-0.22, *N* = 12 *P* = 0.47). Females and extra-pair males tended to mate negatively by body size (tarsus length *r* =-0.52, *N* = 11, *P* = 0.09).

Comparison between extra-pair males and the female's social partner showed that extra-pair males were bigger than the social males (tarsus length: *Z*= -1.39, *N* = 16, *P* = 0.08) and tended to be less colourful (head colour: *Z* = -1.47, *N* = 12, *P* = 0.07), but did not differ in cheek colour (*Z* = -1.00, *N* = 12, *P* = 0.15) or body condition (scaled mass index: *Z* = -1.23, *N* = 16, *P* = 0.10; Figure 3). There was no difference between extra-pair males and social males in their genetic similarity to the female (*Z* = 1.63, *N* = 14, *P* = 0.94; Figure 3).

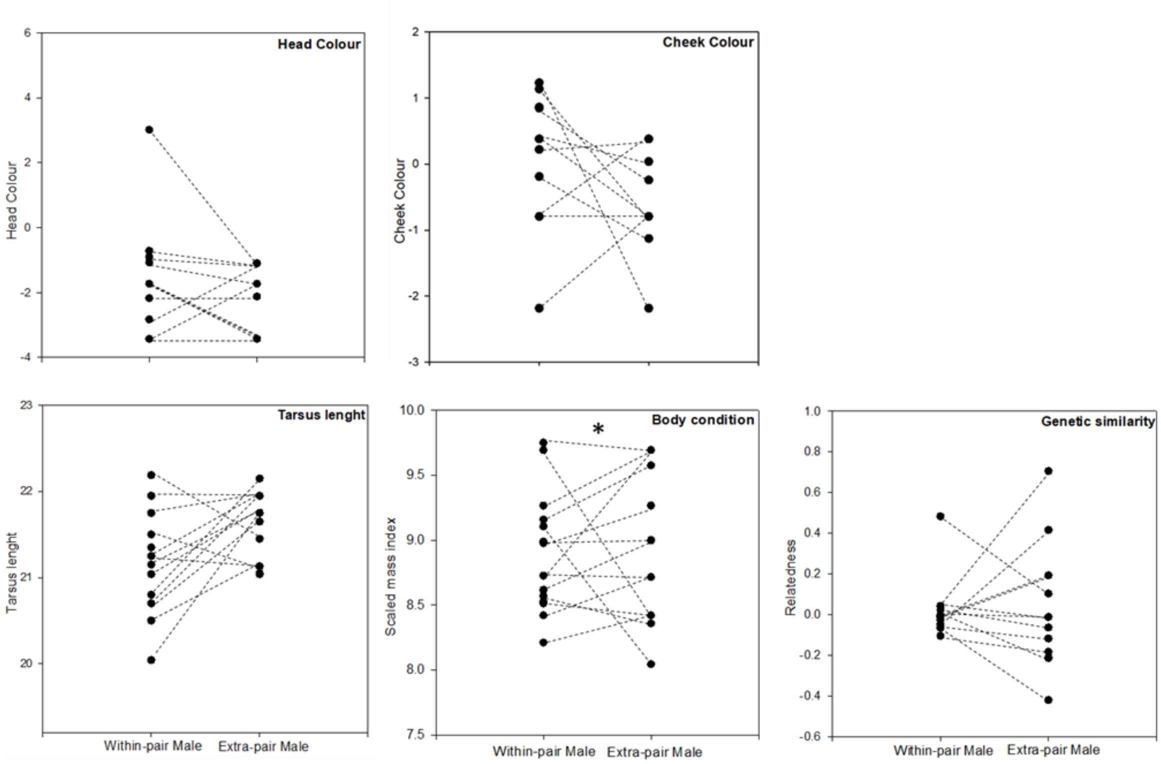

Figure 3. Paired comparisons of different traits of within pair male and extra-pair males. Asterisk indicates statistically significant differences between within-pair and extra-pair males.

Discussion

We investigated whether plumage colouration signalled condition, whether pairs mated assortatively and how plumage colouration influenced female and male reproductive success, female probability to mate extra-pair and male paternity. We found that plumage colour was not related with measures of quality, survival or reproductive success. Females and their social males paired assortatively by plumage colour and parental care, and females also mated assortatively by plumage colour with extra-pair males.

We found that 53% of the nestlings were sired by extra-pair males, and that female and male plumage colour did not predict the proportion of EP offspring, but males gained more paternity in their own nests at low breeding densities. Unexpectedly, we found that males gained greater extra-pair paternity and total paternity when they were less colourful. We also found that extra-pair males were bigger than the males they cuckolded. Taken together, our findings suggest that plumage colour is not consistently related to other measures of male quality, but individuals show assortative mating based on colour, and females (and not males) may exert some mate choice based on colour or less colourful males have intrinsic behavioural differences from more colourful ones.

Plumage colour does not signal condition or incur survival costs

Female and male ornament expression may be used for mate choice, if it provides information about aspects of phenotypic quality, such as body condition (Laczi et al., 2013), parental care ability (Jawor, Gray, Beall, & Breitwisch, 2004), or production of higher quality offspring (Huuskonen, Haakana, & Kekäläinen, 2009). Here, we found no evidence that plumage colour signals body size, condition, or parental care, so it is possible that plumage colouration does not signal direct benefits. However, other as-yet unidentified signalling traits like song could be correlated with these measures of quality. Additionally, plumage could signal other aspects of quality that we did not measure here, such as genetic attributes. For example, females and males may choose according to ‘genetic compatibility” (Brown, 1997), where offspring can benefit by having increased heterozygosity and fitness. Ornaments may express other underlying qualities, such as good genes (Hamilton & Zuk, 1982), immunological capacity (McGraw & Ardia, 2003), age (Dias, Manica, Gressler, Bell, & Fecchio, 2016), or heritable parasite resistance (Doucet & Montgomerie, 2003). These qualities can co-vary with plumage colour, in such way that individuals are choosing compatible or high-quality sexual partners.

Conspicuous colours are often associated with high survival costs, with several studies suggesting that bright colours in birds attract more predation (Götmark & Hohlfält, 1995; Götmark, Post, Olsson, & Himmelmann, 1997; Huhta, Rytkönen, & Solonen, 2003). However, we found that annual survival was unrelated with plumage colouration, suggesting that colourful plumage in the lovely fairy-wren does not incur costs for survival. Supporting this, an experimental study investigating colour phenotypes in fairy-wrens showed that being brightly coloured did not increase the chances of predation (Cain et al., 2019), indicating that increased conspicuousness is not associated with greater predation pressure.

Assortative pairing by colour and parental care

Consistent with the growing body of evidence that individuals mate assortatively with respect to plumage colouration (Griggio et al., 2005; Jacobs et al., 2015; Rowe & Weatherhead, 2011) and parental care (Voltura, Schwagmeyer, & Mock, 2002), we found a similar pattern in the lovely fairy-wrens. Socially monogamous species that contribute similarly to parental care are expected to choose a high quality partner (Fitzpatrick et al., 1995; Safran & McGraw, 2004) and in positive assortative mating, individuals of similar traits are expected to pair more frequently than expected by chance (Burley, 1983). When only one sex is selective, patterns of assortative mating are not expected (Burley, 1983). This mating pattern suggests that plumage colour is expressed in both sexes because it has a signalling function and is favoured by mutual sexual selection (Kraaijeveld et al., 2007). However, competition can also account for the same results if social dominance affects mating (Creighton, 2001). We have shown that plumage colouration is involved in male and female competitive interactions (Leitão et al., *in press*), and thus plumage colouration can reflect the ability of individuals to compete for territories (Hasegawa, Arai, Watanabe, & Nakamura, 2014; Wolfenbarger, 1999b). A similar pattern could arise if individuals chose partners by territory quality and this co-varies with plumage colour. Further, territories with similar resources may produce individuals with similar colour, if plumage colour is resource-dependent. Behavioural observations and experiments are needed to test these ideas.

Female and male breeding success and extra-pair behaviour: correlates with plumage colour?

Males with more conspicuous ornaments are assumed to be of better quality because ornaments may be costly to produce (Hill, 2006). Showy males can be more successful when competing for sexual resources and may be preferred as mates (Andersson, 1994; Darwin, 1871), and so it is expected that these individuals achieve higher reproductive success. Despite finding assortative mating within social pairs and extra-pairs in relation to plumage colour, we found that plumage colouration was not correlated with reproductive success in its home nest. However, females with more colourful plumage tended to have higher reproductive success (Table 5), so we cannot rule out that our measure of breeding performance did not capture a possible relation or effect with plumage colour. For example, individuals that are brighter could have better quality offspring, but general reproductive performance may be masked by predation, the main cause of nest failure in this species (Leitão et al., 2019). It is also possible that bright plumage colours are at a cost for reproduction, due to the trade-off between resources allocated to reproduction vs producing sexual traits (Andersson, 1982; Fitzpatrick et al., 1995). Conspicuous colours may also attract and increase predation at the nest (Wallace, 1889) with some support from correlational and experimental studies (Freeman-Gallant, Schneider, Taff, Dunn, & Whittingham, 2014; Haskell, 1996), which can selectively act against bright colours.

The proportion of EP nestlings in a nest did not show any relation with female plumage colour, but our data suggests that females with helpers were more likely to have extra-pair offspring. This is similar to what was found in superb fairy-wrens *Malurus cyaneus* (Mulder et al., 1994) and splendid fairy-wrens *Malurus splendens* (Webster, Tarvin, Tuttle, & Pruett-Jones, 2004). Cooperative behaviour in *Malurus* has been linked to increased rates of EPP (Brouwer et al., 2017; Mulder et al., 1994; Webster et al., 2004), as females may be liberated by the presence of helpers to engage in extra-pair mating (Mulder et al., 1994), although broad-scale comparative analyses indicate that, across bird species, cooperative breeding is generally associated with low rates of extra-pair paternity (Cornwallis, West, Davis, & Griffin, 2010). In some studies with fairy-wrens it has been found that high and low numbers of helpers have shown the same rates of EPP (Colombelli-Négrel, Schlotfeldt, & Kleindorfer, 2009), suggesting that there may be additional factors influencing paternity.

Against our predictions, we found that less colourful males had higher extra-pair and total (within and extra-pair) paternity success, and were comparatively less colourful than the males they cuckolded. There is evidence from a different fairy-wren species that extra-pair behaviour is under female control (Double & Cockburn, 2000), resulting from female preference for high-quality males. Surprisingly, if this is the pattern, lovely fairy-wren females preferred males that are less colourful, and to our knowledge only one other study found a similar counterintuitive pattern of less colourful individuals having more extra-pair paternity (Delhey, Johnsen, Peters, Andersson, & Kempenaers, 2003).

There are several possible explanations to why less colourful males have higher paternity. First, if females actively seek and compare potential extra-pair males against their social males, even if mating assortatively with the extra-pair by colour, they may be also choosing based on traits that were not quantified in this study, such as song. Second, it could be that structural colours are not costly to produce. Honesty in signals like plumage colours is usually thought to be maintained by either maintenance costs and higher vulnerability to predators (handicap signals; Zahavi, 1975) or production costs, though structural colours do not require extensive physiological investment or nutrients (Prum, Dufresne, Quinn, & Waters, 2009) and evidence for this condition dependency is scarce (Doucet, 2002; Fitzpatrick, 1998; Keyser & Hill, 2000; McGraw, Mackillop, Dale, & Hauber, 2002). Further, it might be that all males engage similarly in pursuing extra-pair matings but less colourful males guard their mates more closely than more colourful ones (Dowling & Webster, 2017), which prevents other males from copulating with their female partner (Birkhead & Møller, 1998) and that decreases the overall EPP success of more colourful males. Additionally, it is possible that less colourful males are bolder, or more aggressive and intrinsically willing to engage in extra-pair behaviour more often (Forstmeier, 2007) or obtain copulations by force (Burg & Croxall, 2006). In a previous study we have shown that less colourful males are more aggressive towards a same-sex (mirror reflection) rival (Leitão et al., *in press*), and here we found that EPP males were bigger than the males that they cuckolded, which is a common trait associated with aggression, dominance (Hagelin, 2002; Raihani, Székely, Serrano-Meneses, Pitra, & Goriup, 2006), and EPP (Green, Peters, & Cockburn, 2002; Wells, Ji, Dale, Jones, & Gleeson, 2014). Alternatively, it might be easier for less colourful males to intrude neighbours' territories if they are considered of lower status, or if they adopt a 'sneaking' strategy (Foote, Brown, & Wood, 1997); in the same line, females may prefer subordinate/less aggressive males because the benefits of mating with this type of male outweigh the costs (Qvarnström & Forsgren, 1998). Why less colourful males are more successful is unclear and whether this pattern is driven by male, female or both remains to be determined.

Lovely fairy-wrens had high rates of extra-pair paternity despite low frequencies of extra-pair courtship displays, high male care, and low number of helpers compared to other *Malurus* (Leitão et al., 2019). Copulations were rarely seen and only within-pair (Leitão et al., 2019), suggesting that females may be seeking extra-pair males in pre-dawn extra-territorial forays, as has been shown for superb fairy-wrens (Double & Cockburn, 2000).

Previous comparative work in the Maluridae family has confirmed that multiple factors such as inbreeding avoidance, the presence of helpers, low levels of male care, and male density drive variation in EPP rates (Brouwer et al., 2017). We found a very similar pattern in this study, since males that were in areas with lower density achieved higher within-pair paternity success, and females tended to have more EP offspring in the presence of helpers in the group. Additionally, all (three) cases of incestuous pairings resulted in EPP (though not all nestlings in these broods were EP), suggesting the possibility that females may partially avoid inbreeding depression by mating extra-pair when partnered with a close relative.

Most studies have focused on male traits and how they affect paternity (Griffith et al., 2002), while little attention has been given to female traits that might influence extra-pair paternity (Moreno et al., 2015), yet EP behaviour is the outcome of female and male interaction (Arnqvist & Kirkpatrick, 2005). Male and female lovely fairy-wrens showed sex differences in their reproductive strategies and its relationship with plumage colouration: while females and males paired and mated assortatively (which suggests mutual choice), no relation was found between female plumage colour and male mate choice, and less colourful males were more successful in getting extra-pair paternity. It is possible that females may be more selective than males and choose social and extra-pair males based on their plumage colour, or mating behaviour in males is related with plumage colour as well as behavioural traits, such as its willingness to foray for extra-pair mates. More empirical work is needed to understand how and what drives females and male mate choice. However, taking these results together with previous work (Leitão et al., *in press*), plumage colour in both sex seems to work in intra and inter-sexual competition contexts and has the potential to shape the evolution of plumage colour.

Acknowledgments

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Appendix

Table A1. Description of microsatellite loci screened for posterior panel, based on x dominant birds.

Locus	N samples run	Nr Alleles	Size range	% Successful samples	Variability
Mcymu1 [1]	22	2	-	0	-
Mcymu4 [1]	35	4	190-196	94.29	0.12
Mcymu6 [1]	35	8	151-161	0.28	0.28
Mcymu7 [1]	230	6	118-126	98.26	0.03
Mcymu8 [1]	230	26	258-353	94.35	0.12
Msp4 [2]	22	3	169-169	0.60	0.60
Msp6 [2]	35	5	279-291	0.17	0.14
Msp10 [2]	35	2	189-189	0.06	0.06
Tmm6 [3]	230	63	316-469	93.48	0.29
Smm1 [4]	208	9	257-273	79.33	0.05
Smm2 [4]	35	4	208-218	0.11	0.11
Smm7 [4]	35	3	262-264	0.09	0.09
Ase9 [5]	13	4	143-149	0.31	0.31
MaLa2 [6]	215	20	203-295	98.60	0.09
MaLa3 [6]	20	3	297-301	0.15	0.15
MaLa4 [6]	215	7	337-353	98.60	0.03
MaLa5 [6]	215	8	193-217	97.67	0.04
MaLa6 [6]	215	7	190-206	98.60	0.03
MaLa7 [6]	215	7	290-310	98.14	0.03
MaLa8 [6]	215	10	233-269	98.14	0.05
MaLa10 [6]	215	11	271-326	98.14	0.08
MaLa13 [6]	215	11	331-363	98.60	0.05
MaLa14 [6]	215	11	250-282	98.14	0.05
MaLa16 [6]	20	4	186-187	0.22	0.22
MaLa18 [6]	215	8	258-290	98.60	0.04

References for microsatellite are: [1](Double et al., 1997); [2](Webster et al., 2004); [3](Adcock & Mulder, 2002); [4](Maguire et al., 2006); [5](Richardson et al., 2000); [6](Thrasher et al., 2017).

Table A2. Correlation of colour measurement between years.

		Year 1	
		Cheek	Head
Year 2	Cheek	r = 0.40, N = 11, p = 0.22	
	Head		r = 0.64, N = 9, p = 0.06

Table A3. General linear model for body condition and body size. Significant predictors in bold. *N* = 34

	Female				Male			
	β	SE	*t*	*P*	*β*	SE	*t*	*P*
Body condition								
Intercept	8.59	0.20	42.50	**<0.001**	8.85	0.08	104.81	**<0.001**
Cheek colour	-0.02	0.10	-0.25	0.80	-0.04	0.06	-0.67	0.50
Head colour	0.03	0.05	0.66	0.51	0.01	0.03	0.27	0.78
Body size								
Intercept	20.44	0.18	112.12	**<0.001**	21.35	0.13	161.14	**<0.001**
Cheek colour	0.12	0.09	1.33	0.19	-0.13	0.10	-1.24	0.223
Head colour	-0.07	0.04	-1.64	0.11	0.03	0.05	0.65	0.51

Table A4. General linear mixed model for survival (1 survived, 0 died). *N* = 74

	β	SE	*z*	*P*
Survival				
Intercept	1.38	1.34	1.03	0.30
Sex	0.20	0.95	0.21	0.83
Cheek colour	0.35	0.37	0.95	0.34
Head colour	-0.01	0.23	-0.05	0.95
Sex * Cheek colour	-0.48	0.62	-0.77	0.43
Sex * Head colour	0.05	0.32	0.18	0.85
Random effect	σ^2			
Year	3.55			

Chapter 5

The form and function of female and male song in Lovely fairy-wrens

Ana V. Leitão, Raoul Mulder, Michelle L. Hall

Abstract

Song in birds has been extensively studied and is considered a classical example of a sexually selected trait. However, this interpretation is based on extensive study of predominantly one sex, the male. There is growing evidence that female song is common and phylogenetically widespread, but there are still only a few species for which song similarities and differences between the sexes have been described. This is necessary to understand what functional and evolutionary differences, if any, exist between females and males in this trait. In this study, we investigated the function of female and male song in the Lovely fairy-wren (*Malurus amabilis*), a tropical species that maintains and defends territories year-round. We analysed song structure and compared song rates of both sexes, through observations across seasons (breeding and non-breeding), breeding stages, and when partners were together or apart. We also tested individual responses to simulated territorial intrusion, using playbacks of female and male songs varying in complexity. Females and males had similar song metrics and natural song rates. Pairs sang more during non-breeding periods than during breeding periods. Within each breeding stage, males sang more than females when females were incubating, and individuals of both sexes sang more when apart than when together. Both females and males responded to simulated intrusion with by singing and approaching the speaker together. Male song playbacks elicited stronger responses than female song playbacks, as did simple playback songs after they were exposed to complex songs, during both breeding and non-breeding periods. Observations and experiments suggest that female and male songs function primarily in joint territorial defence of resources and within-pair communication.

Keywords: female song; female competition; sex differences; song complexity; territory defence

Introduction

Elaborate traits, such as song and plumage colours, have been traditionally seen as male attributes and assumed to have evolved mainly as a result of sexual selection (Andersson, 1994). However, such traits are not expressed solely in males, though female ornaments have often been regarded as rare, unusual or as genetically correlated by-products of selection for ornaments in males (Lande, 1979). Recent growth in the number of studies investigating the function of elaborate traits in females has revealed that they are more common and adaptive in females than previously thought. For example, song in female birds is phylogenetically widespread and ancestral in songbirds, and sexual dimorphism in song may result from recent evolutionary losses of female song, rather than from evolutionary gains of song in males only (Odom et al., 2014).

Female song appears to have comparable functions to male song, including mate attraction (Langmore, Davies, Hatchwell, & Hartley, 1996; Levin, 1996; Morton, Derrickson, & Stutchbury, 2000), mate-guarding (Rogers, Langmore, & Mulder, 2007), intrasexual competition for territories or mates (Grafe & Bitz, 2004; Rogers et al., 2007), group cohesion and coordination (Halkin, 1997; Ritchison, 1983), intra-pair-contact (de Silva, Marantz, & Pérez-Villafaña, 2004), and resource defence (Tobias et al., 2012) (reviewed in Hall, 2004; Langmore, 1998; Odom et al., 2014).

Most of the hypotheses relating to song function were developed for male song and did not consider implications of a singing partner. However, many species have ornaments expressed in both sexes (Kraaijeveld et al., 2007), so it is unclear if female ornaments arise or are maintained by the same selective pressures that were hypothesised for males. Sexual selection alone might not explain the evolution of mutual traits (Kraaijeveld et al., 2007), and it is possible that in both females and males ornament elaboration evolved in competition over reproductive and ecological resources, and so may be better understood through a broader process of social selection (Cain & Rosvall, 2014; West-Eberhard, 1979).

Bird song is a complex acoustic signal, with diverse properties and dimensions that could potentially be assessed by conspecifics (e.g. song complexity, repertoire size, duration, rate, song output). Song attributes may serve as honest indicators of individual quality and be correlated with physical features. For example, in males, features of song such as pitch can be related to body size (Hall et al., 2013; Linhart & Fuchs, 2015) which is an important predictor of success in competition for resources and mate attraction. Repertoire size can indicate brain area and immune capacity (Pfaff, Zanette, MacDougall-Shackleton, & MacDougall-Shackleton, 2007). Like male song, female song can be an indicator trait, e.g. where female song output is related to body size (Mahr, Evans, Thonhauser, Griggio, & Hoi, 2016), song complexity is correlated with age and experience (Keen, Meliza, Pilowsky, & Rubenstein, 2016; Langmore et al., 1996), or song rate predicts breeding success (Brunton et al., 2016; Cain et al., 2015).

The fairy-wrens (Maluridae) have been a model genus for several studies involving song (Buchanan & Cockburn, 2013; Greig et al., 2013). In all fairy-wrens, both females and males sing. The function of song in this family has been explored and found to be important in mate-guarding (Dowling & Webster, 2017), group communication (Payne, Payne, & Rowley, 1988; Payne, Payne, Rowley, & Russel, 1991), dual function in male-male competition and mate attraction (Dalziell & Cockburn, 2008) for territorial defence (Cooney & Cockburn, 1995; Dowling & Webster, 2013), and in particular for joint territorial defence (Hall & Peters, 2008). However, previous studies have mainly focused on one sex, with a few exceptions (Dowling & Webster, 2015; Hall & Peters, 2008; Kleindorfer et al., 2013). Parallel investigations of both sexes are important to draw conclusions about similarities and differences in selective forces shaping female and male ornaments.

Here we examine the form and function of song in the Lovely fairy-wren, *Malurus amabilis*, a cooperative breeder species where both sexes sing but the function of song is unknown. This species is particularly interesting to study because individuals inhabit very dense environments, where long-range signals like song are likely to be important; all-purpose breeding territories are maintained year-round; both sexes participate in territory defence; and males and females have convergent parental roles (Leitão et al., 2019).

We analysed acoustic song structures in both sexes and examined variation in song rates in different social contexts, through observations across and within breeding and non-breeding periods. We then experimentally tested if song complexity of simulated solo female and male intruders triggered different territorial responses. We predicted that if the primary function of song elaboration is mate attraction, song structure would be different between sexes (to advertise/emphasise sexual identity), individuals would sing more during breeding and nest-building periods (fertile stage), and the response to playbacks would be stronger during the breeding season, towards the opposite-sex and to more complex songs (if more complex songs are more attractive: Collins, 2004) (but see Byers & Kroodsma, 2009). By contrast if intra-sexual competition is the key selective pressure shaping song, individuals would be expected to sing year-round rather than during a specific breeding period, and respond more strongly to same-sex playback of more complex songs if these signal a higher threat (Collins, 2004). If partners use song to coordinate movements and jointly defend resources, males and females should have similar song structure, both sexes should sing more when they are apart (i.e. out of visual contact), and both sexes should cooperate to deter intrusion (Logue, 2005).

Methods

Study species and general methods

The Lovely fairy-wren is a year-round territorial resident of the coastal areas of Far North Queensland, Australia, where it inhabits dense mangroves and rainforest edges. The area is characterised by a tropical climate, with marked rainfall seasonality (wet and dry season). Lovely fairy-wrens are socially monogamous and cooperative breeders, although only 37% of groups in these populations have helpers, and these are exclusively male (Leitão et al., 2019). The species is dichromatic and dimorphic, and both males and females sing and defend territories. The species breeds year-round, with a peak in the dry season (August – November) (Leitão et al., 2019). Females build nests and incubate the eggs alone, but all group members take care of nestlings and fledglings, with females and males provisioning nestlings at similar rates (Leitão et al., 2019).

We studied Lovely fairy-wren song behaviour in the wet tropics of Australia in the Cairns area (16.87°S, 145.75°E), in 2015 (May-December), 2016 (March, July-November), and 2017 (March, August-September; for details see Leitão et al., 2019). In brief, birds were captured in mistnets and marked to be individually distinguishable based on a unique combination of colour-bands. After release, pairs or groups were monitored for breeding activity, and nesting attempts were located and followed. Breeding stage was categorised as: not nesting, nest-building, incubating, and provisioning offspring.

Song recordings and song analysis

At capture, birds were subjected to a mirror test (described in Leitão et al., *in press*). The test consisted of two ~5-minute periods, with a hidden mirror exposed during the second period.

We recorded vocal activity throughout the test (and analysed variation in song rates in Leitão et al., *in press*), but for this study we focused on song structure, using only songs sung during the first part of the test, when the mirror was covered. We included 10 – 11 songs each from 16 females and 15 males (see Figure 1 for an example). We used songs recorded during the cage test because of the high recording quality (birds were <1m from microphone throughout). Recordings from free-living birds were of much lower quality because of their very dense habitat, low singing rates and large territories. It is possible that some individuals sang in direct response to the cage test. However, lovely fairy-wrens when agitated, typically respond with chattering or alarm calls (personal observation). Also, our objective was to compare female and male song structure, so we standardised context by recording both sexes under the same conditions.

All recordings were made using a Marantz PMD660 recorder with a Sennheiser ME67 microphone (in 2015), and a ZOOM H5 XYH-5 with an Olympus ME-15 microphone (in 2016).

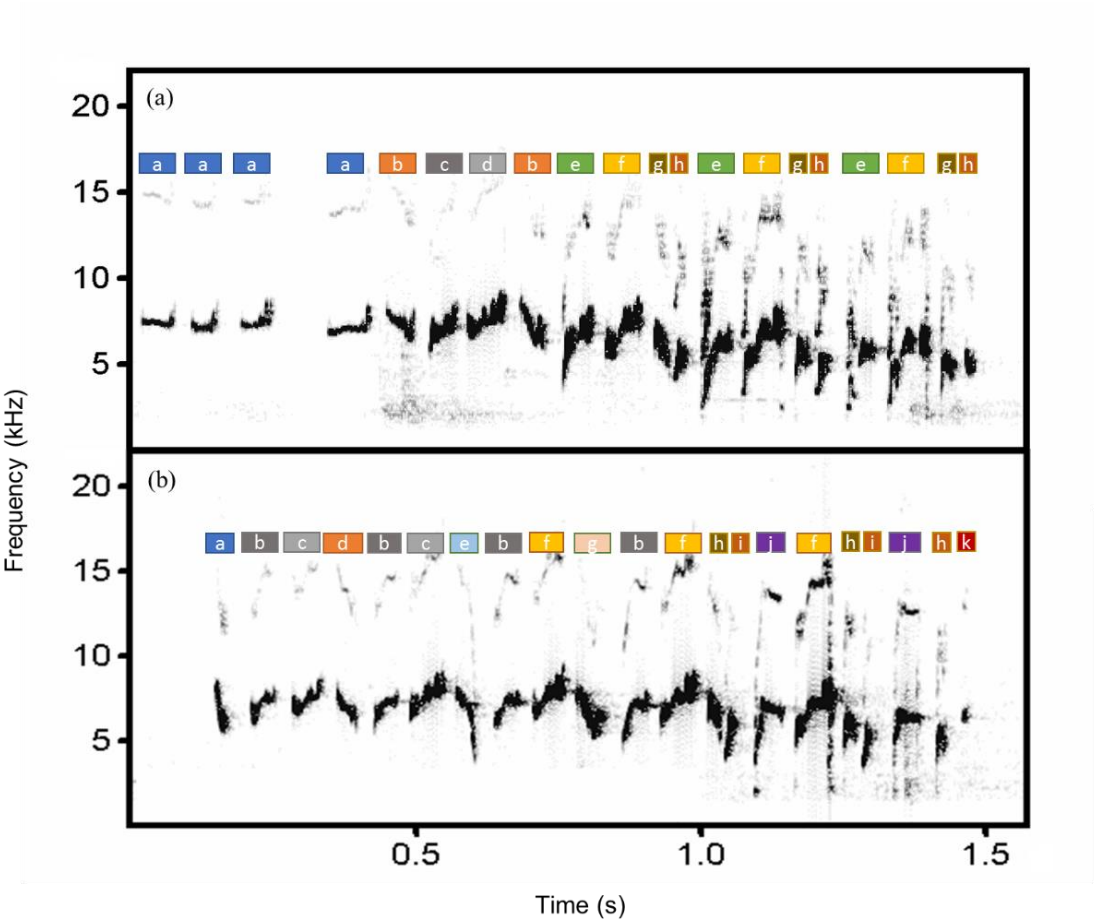

Figure 1. Spectrogram example of female (a) and male (b) Lovely fairy-wren songs. Element types are represented by different colours. Measures of song complexity for this example are: (a) Number of syllable types = 8, total number of syllables = 20, song complexity = 0.40; (b) number of syllable types = 10, total number of syllables = 21, song complexity = 0.47.

We generated spectrograms of the songs from sound files in Avisoft-SASLab Pro v5.2.12 (Avisoft Bioacoustics, Berlin, Germany), with a Hamming window, a 149 Hz bandwidth, a frequency resolution of 86 Hz and a temporal resolution of 2.9 ms (FFT = 512, overlap = 75%). Sound files were high-pass filtered at 1.5 kHz to eliminate low-frequency background noise. We annotated the spectrograms by song, defined as a continuous unit of sound lasting longer than 0.95 s and with no gaps of silence between units for more than one millisecond. A total of six parameters were quantified from each song: song duration (duration from start to end of a song, in seconds), minimum frequency (lowest frequency where the amplitude goes last below 25 dB, in Hz), maximum frequency (highest frequency where the amplitude goes last below the 25 dB, in Hz), peak frequency (the frequency at which the maximum amplitude in the song occurs, in Hz), bandwidth (difference between maximum and minimum frequency, in Hz) and entropy (average randomness or pureness measures for each song). Song duration was measured manually; all other measurements were made using automatic parameter measurement in Avisoft, over the entire song. All automatic measurements were inspected to check for possible error, and background noise that interfered with minimum and maximum frequency measurements was manually eliminated. We averaged measurements across songs for each individual. All measurements were made blind to the sex of the bird.

Spontaneous song behaviour and context

Natural song behaviour (not provoked by experimental playback) was observed through a series of focal observations of 46 groups/pairs between 2015-2017 (more than 400 h). In this analysis, we only included observations from pairs (without subordinates, to eliminate any confounding factor of group size), for which we knew their breeding stage. In total we included ~111h of focal observations on 27 territories (average 2 hours per territory, range: 10 min to 6h14). These observations were made during breeding (July-November) and non-breeding periods (March), between 0552 and 1253 hours, after the dawn chorus. Data from the focal observations was used to investigate natural singing rates for each sex (number of songs per hour) and proximity of the pair when they sang (if partners were within 5 m of one another, or more than 5 m apart), in relation to breeding stage (not nesting, nest-building, incubating, provisioning offspring).

Playback experiment

We performed behavioural experiments by subjecting pairs to simulated territorial intrusions during nest-building (July-December 2016), and during the period where no nesting was observed (March and April 2017). Experiments were conducted in the morning, between 0615 and 1130 hours. We tested each pair with playback simulating either a male or a female intruder. Within each experiment, focal subjects were subjected to two playback treatments, which differed in song complexity, separated by 5 minutes, and presented in random order. In cases where we repeated the experiment in the same group, we presented playback of the two sexes separated by intervals of at least 24h, but no more than 96h. Trials (male vs female, more vs less complex) were balanced in the order in which they were presented with respect to the pair. Altogether, 27 playback experiments were carried out successfully with 19 different pair subjects, and 7 pairs subjects received playback experiments of both sexes. No experiment was conducted on a nearby territory in the same day to avoid habituation.

Stimulus songs used for the playback experiment were selected from the high-quality recordings from the mirror image stimulation test (see '*Song recordings'* above). We created stimuli from 25 individuals (females and males) that were unfamiliar (from more than 2 km away) to the tested pair. To avoid pseudoreplication, each experiment had unique stimuli, except for two stimuli that were repeated in different pairs and at different times of the year (dry and wet season). Playback stimuli were prepared using Avisoft, and we filtered the noise with a 1.5 high pass filter, normalised each song to 90% and saved as uncompressed wave files.

Each experiment consisted of two *ca.* 3 min playback periods, separated by a 5 min period of silence. Each playback period consisted of six songs, interspersed with 20 seconds and 30 seconds of silence (Figure 2), to approximate high natural singing rates (personal observations). Each experiment had two unique songs recorded from the same individual that had similar song duration, and that naturally differed in complexity, one being simpler and the other more complex. Complexity was measured as the number of different elements, divided by the total number of elements per song. Elements were defined as a single continuous trace that did not overlap and were identified according to their appearance (see Figure 1 for terminology). On average simple songs had 6.73 ± 2.26 number of elements, and complex songs had 10.17 ± 2.05 number of elements.

We recorded both vocal and movement responses of both sexes during the experiments using a Marantz PMD660 recorder with a Sennheiser ME67 microphone. Experiments began when both members of the pair were located. After following the birds for 5-10 minutes of undisturbed natural behaviours (or waiting until any disturbance ended, e.g. interactions with neighbours), a loudspeaker (Ultimate Ears Boom 2) was placed approximately 15m from the birds, 1.5m above the ground. Two or more points 5m from the loudspeaker (on either side) were marked with flagging tape to facilitate distance estimation, after which playback commenced. Songs were broadcast at an amplitude of 90 dB at 1 m (Digitech QM-1589 Sound Level Meter). The observer (A.V.L.) stayed quietly among the vegetation 10m from the loudspeaker that broadcast the stimuli and was blinded to the playback type (sex or complexity treatment). Each trial day consisted of a 5-10 min pre-playback observational period, 3 min first playback, 5 min period of observation after playback, 3 min second playback, and further 5 min post-playback period (Figure 2). The observer annotated the recording by dictating female and male distance to each other, the closest approach to the loudspeaker (0, 5m, 10m, or out of 20m area), time spent at each distance, aggressive displays and other behaviours, and identity of singers. No copulation or copulation solicitation was observed during the experiments. In one case where neighbours interfered, the playback experiment was interrupted.

We used the recordings and annotated observations to extract the following responses for each sex during playback trials: i) number of songs; ii) latency to sing the first song; iii) closest approach to speaker (m); iv) latency to approach within <5m from the speaker. We also recorded the v) total time of response to the playback, considering the first approach or song in response to the playback until the last song or if bird left the area within 10m, including post-playback period. All information was extracted from audio records of the experiment blind to the treatment type.

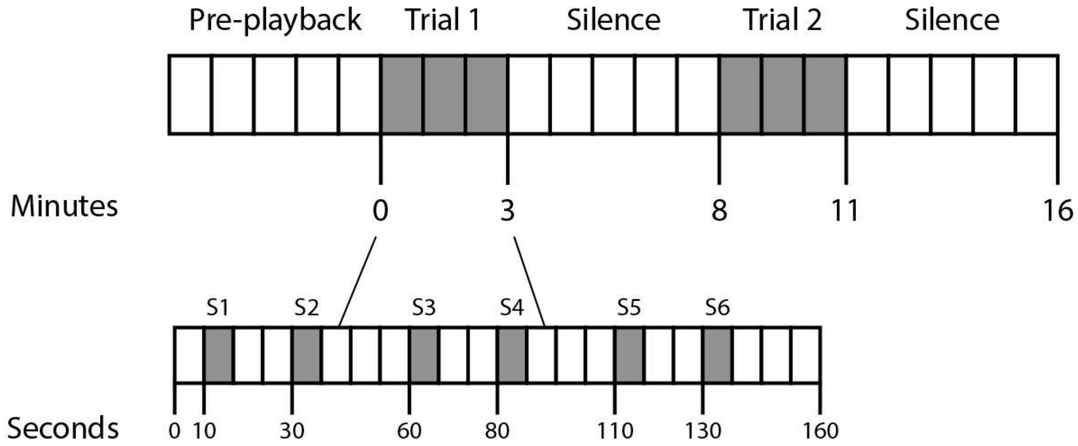

Figure 2. Playback scheme. Each experiment lasted approximately 16 minutes (top). Within each experiment a trial consisted of playback of 6 songs (bottom).

Statistical analysis

Statistical analyses were performed in R v3.4.3 (The R Foundation for Statistical Computing). We used a one-way analysis of variance (ANOVA) of individual means for each acoustic song parameter to test if there were song structure differences between sexes.

To determine which factors influence female and male natural song rates, we used a general linear mixed model (GLMM) with normal error distribution, with log transformed song rate (number of songs per hour) as the dependent variable. We assessed the effect of sex of the focal individual on song rate and its interaction with breeding stage (not breeding, building, laying, incubating, feeding offspring), time since sunrise and day of season (since 1 July). Individual ID was included as a random effect to account for multiple measures of the same individual and Pair ID to control for multiple estimates for each pair. To determine the differences between breeding stages, we used *post hoc* Tukey test comparisons among the least-squares means, using lsmeans package (Lenth, 2016). To analyse the factors related to the proximity of singing partners, we analysed the proportion of songs sung when partners were together (vs apart), using a GLMM with binomial error distribution, and with the same predictors and random factors as the first model for song rate.

To determine whether response to playback within pair was coordinated, we examined the correlation between female and male singing and physical approach responses with spearman correlation coefficients. We then summarised the overall singing and physical approach responses of individuals to the playback experiment with a principal component analysis (PCA), which resulted in two factors, PC1 and PC2, that together explained more than 90 % of the variation (Table 1). PC1 corresponded to ‘approach response’, with high positive values indicating quick approach, approaching closest to the speaker, and more time spent responding to the playback. PC2 corresponded to ‘song response’ where high and positive values indicated more time spent singing and quickly singing the first song (Table 1). To analyse the response to playback, we used a GLMM with a normal distribution and PC1 ‘approach response’ or PC2 ‘song response’ as the dependent variables. We assessed effects of sex of the focal bird, playback treatment (more complex, less complex) and interaction with playback sex (male, female), and the interaction between sex and playback treatment and playback sex, to analyse possible sex differences. In addition, we controlled for other variables: trial order (order of presentation: Complex-Simple vs Simple-Complex), interaction between treatment and trial order, day of trial (first or second day), time since sunrise, day of season, and season (wet, dry season). Pair ID and Individual ID were included as random factors to account for non-independence of the data from up to 4 trials (simple vs complex female songs, simple vs complex male songs) conducted on each individual and pair.

In all models, covariates were centered and standardised. Non-significant terms were sequentially removed from the models by stepwise backward procedures, starting with interactions terms ($P > 0.2$ were removed). We also present full models for comparison in the Appendix. GLMMs were performed using packages lme4 (Bates & Maechler, 2010) and the significance of factors and degrees of freedom (Satterthwaite’s method for approximating degrees of freedom) were assessed using the lmerTest package (Kuznetsova et al., 2017). We report the mean ± SD for descriptive statistics throughout.

Table 1. Loadings for the principal component analysis (PCA) of behaviours in response to the playback experiment.

	PC1 “approach response”	**PC2 “song response”**
Number of songs	0.36	0.59
Latency for the first song	-0.33	-0.62
Closest approach to the playback	-0.51	0.26
Latency to approach within 5m	-0.50	0.27
Total duration of response	0.47	-0.32
Eigenvalues (% of Variance)	1.73 (60%)	1.23 (30%)

Results

Song structure differences between sexes

Female and male song did not differ in any parameters measured (Table 2), though males tended to have lower maximum frequency compared to females.

Table 2. Measurements of song structure from females and males. Statistical values are for comparison between sexes and were calculated using ANOVA except for Entropy (*), which was calculated using Mann-Whitney statistical test.

Parameter	Female songs (*N* = means for 16 females)			Male songs (*N* = means for 15 males)			*F*	*P*
	Min	Max	Mean ± s.d.	Min	Max	Mean ± s.d.		
Song duration (s)	1.05	1.98	1.41 ± 0.25	0.946	1.722	1.308 ± 0.23	1.42	0.24
Peak frequency (Hz)	5119	7488	6539 ± 532	5610	6987	6438 ± 334	0.36	0.54
Min. frequency (Hz)	2552	4646	3438 ± 652	1959	3829	3126 ± 445	2.24	0.14
Max. frequency (Hz)	8693	10176	9495 ± 412	8471	9814	9249 ± 375	2.81	0.10
Bandwidth (Hz)	4972	7461	6051 ± 681	5464	7073	6118 ± 433	0.09	0.75
Entropy	0.38	0.47	0.42 ± 0.02	0.38	0.46	0.42 ± 0.02	0.72*	0.76

Spontaneous song rates and context

Mean female song rate was 6.76 ± 14.65 songs/h and mean male song rate was 10.95 ± 17.30 songs/h. Male and female song rates were statistically similar overall (t = -0.01, P = 0.99), though males had higher song rates than females during incubation (Sex * Incubating, Table 3 (a) and in the full model Table A1). Individual song rates were significantly higher when not nesting compared to all other breeding stages (Table 3 (a), Fig. 3, Table A2), but not different between the different breeding stages (post-hoc analysis: Table A2). Males tended to have higher song rates than females earlier in the day (Sex * Time from sunrise, Table 3(a)).

On average 72 ± 39 % female songs and 65 ± 38 % of male songs were given when apart from their partner. Males sang a higher proportion of songs when together with their partner than females (Table 3 (b), Fig. 4), and both sexes sang more together when not nesting comparing to nest-building and incubating breeding stages (Table, 3 (b), Fig. 4). Pairs sang a higher proportion of songs when they were together earlier in the season. Females (and males tended to) sang fewer songs when together with their partner earlier in the day (Sex * Time from sunrise, and marginal to significance for Time from sunrise, Table 3 (b)).

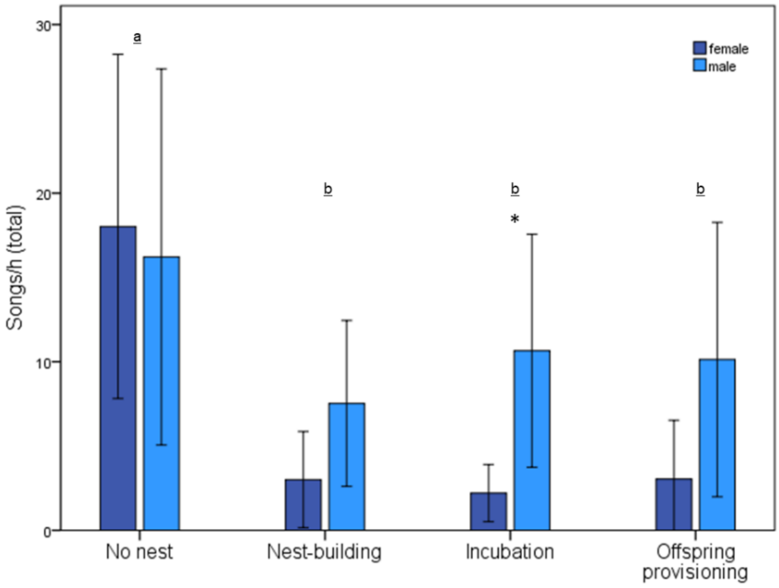

Figure 3. Singing rate of females and males at different breeding stages. Bars indicate the mean and whiskers the standard error. Different letters above bars indicate statistical significance between breeding stage; asterisk indicates statistical significance between sexes.

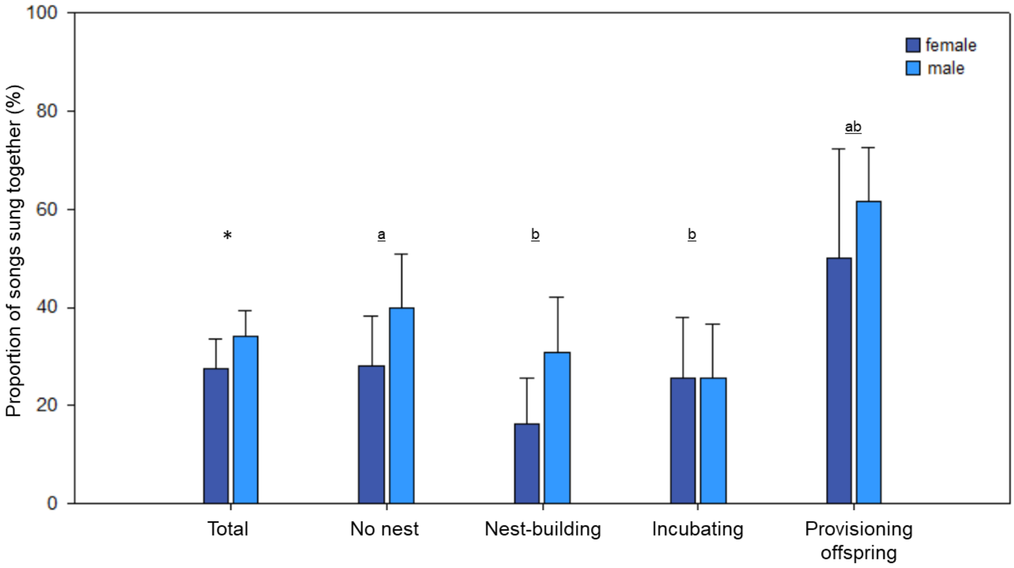

Figure 4. Proportion of female versus male songs sung when partners were together (rather than apart), in total, and across breeding stages. Bars indicate the mean and whiskers the standard error. Different letters above bars indicate statistically significant differences between breeding stages; asterisk indicates statistically significant differences between the sexes.

Table 3. Predictors of (a) song rate and (b) proportion of songs sung when partners were together (vs apart). Significant predictors in bold, marginal to significance italicised. See full models in the Appendix. Breeding stages: no nest (*N* = 41), nest-building (*N* = 48), incubating (*N* = 48), provisioning offspring (*N* = 26).

	β	SE	df	*t*	*P*
(a) Song rate (observations N = 163)					
Reduced Model					
Intercept	**0.92**	**0.12**	**111.32**	**7.67**	**<0.0001**
Sex^1	-0.001	0.15	78.59	-0.01	0.99
Breeding stage^2					
Nest-building	**-0.60**	**0.14**	**141.95**	**-4.10**	**<0.0001**
Incubating	**-0.65**	**0.15**	**150.81**	**-4.12**	**<0.0001**
Provisioning offspring	**-0.71**	**0.18**	**151.88**	**-3.97**	**<0.001**
Time from sunrise	-0.02	0.111	139.15	-0.20	0.83
Sex^1 * Breeding stage^2					
Sex * Nest-building	0.29	0.20	118.32	1.48	0.14
Sex * Incubating	**0.51**	**0.20**	**101.65**	**2.47**	**0.01**
Sex * Provisioning offspring	0.25	0.23	104.93	1.08	0.28
Sex^1 * Time from sunrise	0.26	0.14	109.13	1.80	*0.07*
Random Effect	σ^2				
Individual ID (*N* = 60)	0.01				
Pair ID (*N* = 36)	0.06				
Residual	0.20				
(b) Proportion of songs sung together (vs apart) (observations N = 161)					
Reduced Model					
Intercept	-0.23	0.54		-0.43	0.66
Sex^1	**0.61**	**0.30**		**1.99**	**0.04**
Breeding stage^2					
Nest-building	**-2.07**	**0.53**		**-3.89**	**<0.0001**
Incubating	**-3.13**	**0.66**		**-4.70**	**<0.0001**
Provisioning offspring	-0.35	0.76		-0.46	0.64
Time from sunrise	1.04	0.60		1.72	*0.08*
Day of season	**-1.53**	**0.46**		**-3.29**	**<0.001**
Sex^1 * Time from sunrise	**-1.84**	**0.69**		**-2.66**	**<0.01**
Random Effect	σ^2				
Individual ID (*N* = 60)	0				
Pair ID (*N* = 35)	3.32				
Residual					

Categorical terms: ^{1} Sex reference is female; ^{2} Breeding stage has 4 levels – no nest, nest-building, incubating, provisioning offspring and reference is no nest. Backward step-wise procedures were used to remove non-significant variables (P > 0.2), see Appendix for full model.

Playback experiment

Simulated territorial intrusions elicited rapid and close approaches, short latencies to singing and high song rates (Table 2). Within-pair response was highly correlated for all variables (number of songs: $r = 0.53$ $P = 0.002$; latency for the first song: $r = 0.36$, $P = 0.04$; closest approach to the playback: $r = 0.83$, $P < 0.0001$; latency to approach within 5m: $r = 0.89$, $P < 0.0001$).

Females and males typically approached the speaker together (69% of 39 trials), with similar intensity and more strongly to male playbacks than female (Table 4 (a), Figure 5). There was also an order effect, with pairs approaching the first trial more than the second. Males and females tended to respond more strongly during the wet season (non-breeding season, Table 4 (a)).

Females sang on average 0.19 songs during playback (3.87 ± 11.82 songs/h), and males sang 0.38 songs (7.74 ± 15.80 songs/h), lower than their natural rates (females: $Z = -3.00$, $P = 0.001$; males: $Z = -3.95$, $P = 0.002$). Females and males sang with shorter latency and at higher rates to simple (than complex) songs but, only when they were presented second, that is, after complex songs (PC2: Treatment * Trial order, Table 4 (b); Figure 6; post-hoc analysis: Table A4). Pairs that were subjected to two days of trial responded more strongly on the first day than the second (Table 4 (b)).

Table 4. Effect of predictors on (a) 'Approach response' and (b) 'Song response'. Observation *N* = 62. Reduced models are shown with significant predictors in bold, marginal to significance italicised. See full models in the Appendix.

	β	SE	df	*t*	*P*
(a) PC1 'Approach response'					
Reduced model					
Intercept	-0.73	0.55	27.34	-1.33	0.19
Sex[1]	0.52	0.33	14.63	1.56	0.13
Playback sex[2]	**1.00**	**0.37**	**46.15**	**2.71**	**<0.01**
Season[3]	0.89	0.53	52.94	1.67	*0.09*
Trial order[4]	**-0.93**	**0.24**	**35.23**	**-3.75**	**<0.001**
Time from sunrise	0.95	0.59	32.88	1.59	0.11
Sex[1] * Season[3]	-0.60	0.44	21.16	-1.33	0.19
Random Effect	σ^2				
Individual ID (*N* = 30)	0.02				
Pair ID (*N* = 15)	2.14				
Residual	0.71				
(b) PC2 'Song response'					
Reduced model					
Intercept	0.04	0.36	55	0.131	0.89
Sex[1]	0.41	0.28	55	1.44	0.15
Treatment[5]	-0.48	0.43	55	-1.10	0.27
Trial order[4]	-0.26	0.41	55	-0.64	0.52
Day of trial[6]	**-0.69**	**0.33**	**55**	**2.04**	**0.04**
Time from sunrise	-0.48	0.29	55	-1.60	0.11
Treatment[5] * Trial order[4]	**1.34**	**0.58**	**55**	**2.27**	**0.02**
Random Effect	σ^2				
Individual ID (*N* = 30)	0.00				
Pair ID (*N* = 15)	0.00				
Residual	1.29				

Categorical terms: [1]Sex reference is female; [2]Playback sex reference is female playback. [3] Season reference is dry season. [4] Trial order reference is the first order of presentation (1).[5] Treatment has 2 levels – complex and simple songs treatment and reference is complex treatment. [6]Day of trial reference is first day of the experiment. Backward step-wise procedures were used to remove non-significant variables (P > 0.2), see Appendix for full model.

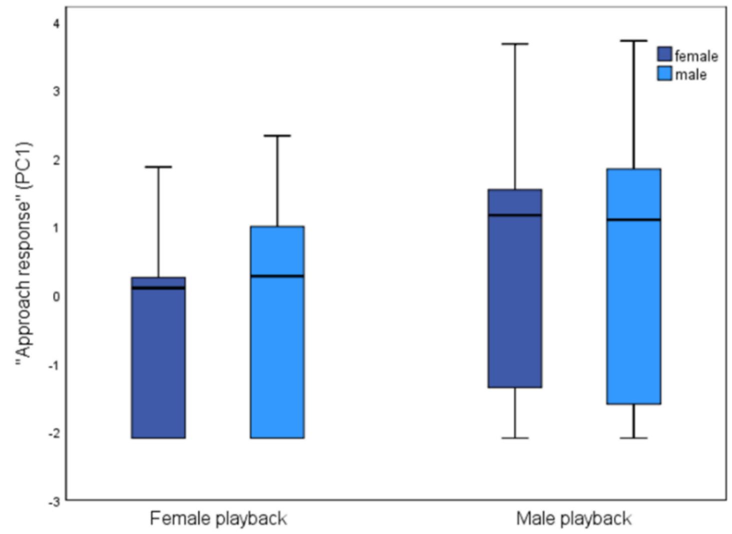

Figure 5. Approach response to territorial simulated intrusion according to sex. Box plot shows the median and the interquartile range, whiskers indicate the values within 1.5 times the interquartile range.

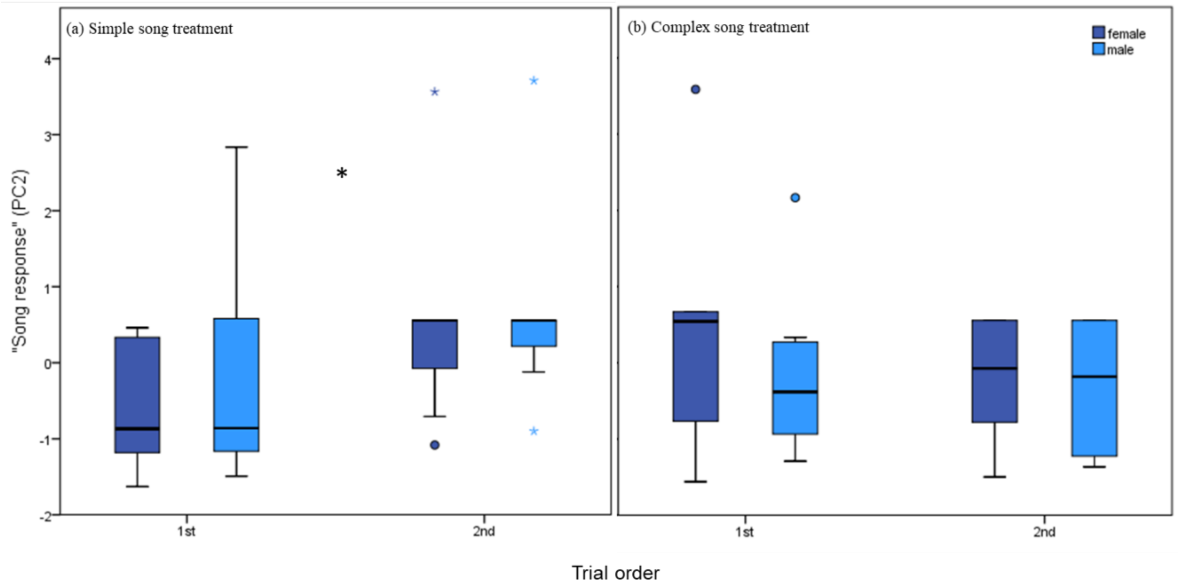

Figure 6. Song response according to complexity treatment and trial day. Black asterisk indicates statistical significance between trial order in the simple song treatment. Box plot shows the median and the interquartile range, whiskers indicate the values within 1.5 times the interquartile range and circles or asterisks are outliers.

Discussion

We compared female and male song structure, song contexts and used a playback experiment to simulate a territorial intrusion, in order to distinguish between different possible functions of female and male song. Female and male lovely fairy-wrens had monomorphic songs and similar song rates across breeding and non-breeding events, except for during the incubation period. Pairs sang more when not nesting compared to all breeding stages, and when apart from their partner. Both sexes had coordinated and similar responses to the intrusions, being more responsive to male playbacks, and to simple songs when these were followed by complex song playback. Taken together, these results suggest that song is used for joint territorial defence and within-pair communication.

Similarities between sexes in song parameters and song rate

Female and male song parameters were similar across all metrics measured, suggesting that their songs are monomorphic. Similar to our results, in the streak-backed oriole *Icterus pustulatus* (Price et al., 2008), superb starlings *Lamprotornis superbus (Pilowsky & Rubenstein, 2013),* pied bushchats *Saxicola caprata* (Sethi, Bhatt, & Kumar, 2012), and the closely related splendid fairy-wren *Malurus splendens* and variegated fairy-wren *Malurus lamberti* (Colombelli-Négrel, 2016), females and males have similar acoustic structure. Other studies have found that species can also exhibit distinctive sex-specific songs. For example, in Venezuelan troupial *Icterus*, females have higher maximum frequency and shorter songs compared to males (Odom et al., 2016); or in the superb fairy-wren *Malurus cyaneus*, females produced shorter songs with higher minimum frequency (Kleindorfer et al., 2013). It is possible that other metrics not measured here, like song complexity or repertoire size, are distinctive between sexes, as seen in other species (Brunton & Li, 2006; Geberzahn & Gahr, 2011; Hall, Rittenbach, & Vehrencamp, 2015; Pavlova, Pinxten, & Eens, 2005; Sethi et al., 2012). Since our results showed a different response towards playback from unfamiliar males and females, individuals were able to discriminate acoustically on the basis of sex, and additional studies are needed to address the song features they used to distinguish sex.

Females and males sang year-round and overall with similar song rates. In some species, females sing as much or more than males (Illes & Yunes-Jimenez, 2009; Pilowsky & Rubenstein, 2013; Price et al., 2008), but most studies so far show that female song is less frequent than male song (Austin et al., 2019; Dingle & Slabbekoorn, 2018; Hall et al., 2015; Krieg & Getty, 2016), including the closely-related purple-crowned fairy-wren *Malurus coronatus* (Hall & Peters, 2008). Overall, females and males appear to produce songs that are similar in form and context, which suggests that song may function in similar manner in both sexes.

Context dependence in song

If song functions for mate attraction, females may sing to advertise fertility to partners or extra-pair males (Mace, 1987), and males to advertise their quality to extra-pair females (Collins, 2004), an hypothesis that predicts that females should sing more when they are fertile (nest-building period) than any other period or non-nesting, while males should sing more during breeding than non-breeding periods. When comparing sexes through different breeding and non-breeding periods, female and male song rates were also similar, except during incubation, where males sang more than females. During incubation the female is naturally more often apart from her partner, since female takes sole care of incubation and bouts can last up to 1 h (Leitão et al., 2019). Indeed, when looking at proportion of songs that individuals sang when together or apart, both females and males sang more when they were apart from their partner during nest building and incubation. This could indicate that individuals use song to advertise quality or fertility to their partner or even to extra-pair individuals, since lovely fairy-wrens have high EPP levels (53 % of offspring are a result of extra-pair mating, Chapter 4). However, as males and females maintained relatively low levels of song rate during breeding compared to the non-nesting period, this suggests that song is not primarily used for mate attraction. It is more likely that individuals may use song to maintain contact with their partner across large territories (1.7 ha on average Leitão et al., 2019). Observations showed that individuals spent most of their time within 5 m to each other, and when not in visual contact, they would call and increase their song rate (AVL personal observations). Song may allow pairs to maintain contact in their dense foliated habitat where visual signals are less effective (Logue, 2007; Mennill & Vehrencamp, 2008). Within-pair communication may also assist in pair-coordination to reduce nest predation (Raihani, Nelson-Flower, Moyes, Browning, & Ridley, 2010), or in response to territorial threats (Hall & Magrath, 2007; Hall & Peters, 2008).

Pairs sang more when they were not breeding, compared to any other breeding stages and were similarly responsive to simulated territorial intrusions across different seasons, but tended to respond more strongly during the non-breeding period. This suggests that song and territorial defence plays a more important role outside the breeding period. During this period, territory holders may encounter higher challenges from prospecting dispersers, and song and aggression can be used to advertise territory ownership. Song can also function to maintain contact between partners, that may increase when coordination associated with joint nesting activities is reduced. However, lower song rate during breeding season may also reflect energetic constraints or risk of predation (Gil & Gahr, 2002) due to nest-building and offspring provisioning. In superb fairy-wrens, female song rate near the nest predicted nest predation (Kleindorfer et al., 2016), which might act as a selective pressure for reduced female song (and males when parental roles are similar) during breeding. Regardless of the drivers of song and territorial defence during non-breeding periods, both males and females are likely to use song as signal for shared defence of resources (see below).

Coordination and joint territorial defence

Lovely fairy-wrens maintain territories year-round (Leitão et al., 2019) and here we show that this includes actively defending territories against intruders during both breeding and non-breeding periods. Pairs responded to intrusion in a coordinated and similar manner, suggesting that pairs defend their territories cooperatively. Earlier studies have shown similar correlated within-pair responses to a simulated territorial intrusion including fairy-wrens (Colombelli-Négrel, 2016; Enbody et al., 2018; Hall & Peters, 2008; Kleindorfer et al., 2013), and in highly coordinated duetting species such as the yellow-naped amazon *Amazona auropalliata* (Dahlin & Wright, 2012), and riverside wren *Cantorchilus semibadius* (Quirós-Guerrero, Janeiro, Lopez-Morales, Cresswell, & Templeton, 2017).

Both sexes approached more quickly and closely towards simulated male intruders. Our results are in line with other studies in superb fairy-wrens (Kleindorfer et al., 2013), and in variegated fairy-wrens (Colombelli-Négrel, 2016), though most studies have shown a sex-specific response, with stronger responses to same-sex intruders (Brunton, Evans, Cope, & Ji, 2008; Fedy & Stutchbury, 2005; Hall, 2000; Rogers, Mulder, & Langmore, 2006) (reviewed in Logue, 2005). A sex-specific response to same-sex playback intruders can signal competition over mates or same-sex resources (Cain & Langmore, 2015; Mays Jr & Hopper, 2004), whereas a coordinated and similar response can convey a joint territorial defence over similar ecological and social needs (Logue, 2005; West-Eberhard, 1979). Females and males may share mutual benefits in defending territories against intruders, and joint defence is possibly more effective at deterring intruders (Langmore, 1998; Logue, 2005). Studies have shown that individuals often respond more strongly to paired intrusions (Demko & Mennill, 2018) and to duets than solo songs (Dowling & Webster, 2015; Odom & Omland, 2018), suggesting that two birds singing are more threatening than one (but see Bradley & Mennill, 2009; Seddon & Tobias, 2005).

The fact that both sexes responded more strongly towards male playbacks, suggests that male intruders represent a greater threat than females. Disputes between residents are frequently observed at the edge of their territories (Leitão et al., 2019), but intrusions into neighbouring territories are commonly made by males (female intrusions were rarely observed, AVL personal observations), which makes males a normal challenge. Also, since males are philopatric (Leitão et al., 2019), an intruding male can be a competitor for territory ownership and consequently a higher threat. Alternatively, females may have responded more strongly to intruding males for potential mating, while male partners were mate-guarding, although we would expect females to display copulation solicitation (and no aggressive displays), and males to disrupt female responses, which we did not observe during the playback experiments.

We also found that pairs responded more quickly and sang more in response to playback of simple songs, but only when they had experienced a complex song in the preceding treatment (treatment effect interacted with trial order). These results indicate that lovely fairy-wrens can decode variation in song complexity, and complexity might be used as a signal. The empirical evidence is mixed, and different species appear to differ in whether less or more complex songs are used in aggressive contexts (Collins, 2004). For example, male tropical mockingbird *Mimus gilvus* were more responsive to less complex male songs (Botero & Vehrencamp, 2007), but male chaffinches *Fringilla coelebs* responded more strongly to more complex male songs (Leitao, Ten Cate, & Riebel, 2006). In the present study, it is possible that complex songs are perceived as more threatening and so residents are less likely to challenge them; and when hearing a less threatening simple song after a complex one, pairs are more encouraged to attack. To our knowledge, this is the first study and evidence for female and male responses to female and male song complexity, and we recommend future studies to address song complexity in both sexes, which may help determine how widespread these results are.

Taken together, the matching response of both females and males towards male simulated intruders and simple songs shows that partners engage in joint territorial defence. Given that the responses were similar outside the breeding period, these findings support the idea of selection on song to defend ecological resources (Tobias et al., 2012).

Conclusion

In species that occupy territories and breed year-round, territorial defence is vital since territories provide resources for reproduction and survival. In the tropics, habitat may be saturated and competition for partners or territories can be intense (Slater & Mann, 2004; Stutchbury & Morton, 2001). The low adult mortality and in consequence low territory turnover observed in this species (Leitão et al., 2019), suggests there is a high territorial pressure. Our study adds to the growing body of work supporting the hypothesis that song elaboration and aggression in females is more likely to evolve in the context of territory or resource competition than for mate attraction (Cain & Rosvall, 2014; LeBas, 2006; Tobias et al., 2012). Future work should examine how song relates to resources such as territories, to infer the selective pressures driving the expression of ornaments.

Acknowledgements

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Appendix

Table A1. Effect of fixed predictors on (a) song rate and comparison between breeding stages and (b) proximity between pair when singing. Significant predictors in bold, marginal to significance italicised.

Fixed effects	β	SE	df	t	P
(a) Song rate (observations $N = 163$)					
Full Model					
Intercept	**1.01**	**0.14**	**107.46**	**7.19**	**<0.001**
Sex[1]	-0.07	0.19	86.693	-0.39	0.69
Breeding stage[2]					
Nest-building	**-0.71**	**0.17**	**149.49**	**-4.10**	**<0.001**
Incubating	**-0.79**	**0.19**	**147.22**	**-4.00**	**<0.001**
Provisioning offspring	**-0.82**	**0.20**	**146.50**	**-4.04**	**<0.001**
Time from sunrise	-0.02	0.11	138.20	-0.24	0.80
Day of season	-0.17	0.14	150.86	-1.17	0.24
Sex[1] * Breeding stage[2]					
Sex * Nest-building	0.38	0.24	115.99	1.57	0.11
Sex * Incubating	**0.62**	**0.27**	**102.86**	**2.30**	**0.02**
Sex * Provisioning offspring	0.34	0.27	100.54	1.24	0.21
Sex * Time from sunrise	0.27	0.15	101.55	1.77	*0.07*
Sex * Day of season	0.13	0.19	110.85	0.66	0.50
Random Effect	σ^2				
Individual ID ($N = 60$)	0.01				
Pair ID ($N = 36$)	0.05				
Residual	0.21				
(b) Proportion of songs sung together (vs apart) (observations $N = 161$)					
Full Model					
Intercept	-0.42	0.60		-0.70	0.48
Sex[1]	0.76	0.48		1.57	0.11
Breeding stage[2]					
Nest-building	**-2.22**	**0.80**		**-2.77**	**< 0.01**
Incubating	**-2.46**	**0.95**		**-2.58**	**<0.01**
Provisioning offspring	0.89	1.25		0.70	0.47
Time from sunrise	**1.29**	**0.66**		**1.94**	**0.05**
Day of season	-0.93	0.64		-1.45	0.14
Sex[1] * Breeding stage[2]					
Sex * Nest-building	0.21	0.84		0.25	0.79
Sex * Incubating	-0.81	0.86		-0.93	0.34
Sex * Provisioning offspring	-1.48	1.28		-1.15	0.24
Sex * Time from sunrise	**-2.07**	**0.74**		**-2.78**	**<0.01**
Sex * Day of season	-0.95	0.70		-1.35	0.17
Random Effect	σ^2				
Individual ID ($N = 60$)	0.0001				
Pair ID ($N = 35$)	3.6				
Residual					

Categorical terms: [1] Sex reference is female sex; [2] Breeding stage has 4 levels – no nest, nest-building, incubating, provisioning offspring and reference is no nest.

Table A2. Comparison between breeding stages and song rate. Results from Tukey method for post hoc comparisons among the least-squares means treatments. Breeding stages: no nest (*N* = 41), nest-building (*N* = 48), incubating (*N* = 48), provisioning offspring (*N* = 26). Significant predictors in bold.

Treatment Pairwise comparison	β	SE	df	*t*	*P*
no nest – nest-building	**0.45**	**0.11**	**136.81**	**4.14**	**<0.001**
no nest - incubating	**0.40**	**0.12**	**124.35**	**3.21**	**<0.01**
no nest – provisioning offspring	**0.60**	**0.14**	**128.71**	**4.23**	**<0.001**
nest-building - incubating	-0.05	0.11	130.87	-0.44	0.97
nest-building – provisioning offspring	0.14	0.13	133.42	1.09	0.69
incubating – provisioning offspring	0.19	0.13	130.43	1.46	0.46

Table A3. Effect of fixed predictors on playback response. Significant predictors in bold, marginal to significance italicised. Observations *N* = 62.

	β	SE	df	*t*	*P*
(a) PC1 ‘Approach response’					
Full Model					
Intercept	-5.05	5.36	12.14	-0.94	0.36
Sex^1	0.53	0.44	35.69	1.20	0.23
Playback sex^2	0.91	0.54	47.87	1.67	*0.09*
Treatment^3	-0.50	0.78	37.34	-0.64	0.52
Season^4	9.43	9.78	12.28	0.96	0.35
Trial order^5	-1.20	0.65	26.33	-1.85	*0.07*
Day of trial^6	-0.12	0.39	47.68	-0.31	0.75
Time from sunrise	0.90	0.64	26.30	1.40	0.17
Day of season	-8.68	9.75	12.47	-0.89	0.39
Sex^1 * Playback sex^2	-0.20	0.50	35.69	-0.41	0.68
Sex^1 * Treatment^3	0.10	0.48	35.69	0.22	0.82
Sex * Season^4	-0.57	0.49	35.69	-1.15	0.25
Playback sex^2 * Treatment^3	0.11	0.54	37.73	0.21	0.83
Treatment^3 * Trial order	0.40	1.17	19.34	0.34	0.73
Random Effect	σ^2				
Individual ID (*N* = 30)	0.00				
Pair ID (*N* = 15)	2.18				
Residual	0.82				
(b) PC2 ‘Song response’					
Full Model					
Intercept	0.651	2.35	48.00	0.27	0.78
Sex^1	0.57	0.58	48.00	0.97	0.33
Playback sex^2	-0.32	0.57	48.00	-0.56	0.57
Treatment^3	-0.74	0.66	48.00	-1.11	0.26
Season^4	-0.74	4.40	48.00	-0.17	0.86
Trial order^5	-0.30	0.44	48.00	-0.67	0.50
Day of trial^6	-0.68	0.38	48.00	-1.77	*0.08*
Time from sunrise	-0.51	0.32	48.00	-1.57	0.12
Day of season	0.83	4.40	48.00	0.18	0.85
Sex^1 * Playback sex^2	0.13	0.66	48.00	0.19	0.84
Sex^1 * Treatment^3	0.02	0.63	48.00	0.03	0.97
Sex * Season^4	-0.41	0.65	48.00	-0.63	0.52
Playback sex^2 * Treatment^3	0.52	0.65	48.00	0.81	0.42
Treatment^3 * Trial order	**1.40**	**0.63**	**48.00**	**2.22**	**0.03**
Random Effect	σ^2				
Individual ID (*N* = 30)	0.0				
Pair ID (*N* = 15)	0.0				
Residual	1.43				

Categorical terms: ^{1}Sex reference is female; ^{2}Playback sex reference is female playback. ^{3}Treatment has 2 levels – complex and simple songs treatment and reference is complex treatment. ^{4}Season reference is dry season. ^{5}Trial order reference is the first order of presentation (1). ^{6}Day of trial reference is first day of the experiment.

Table A4. Comparison between the interaction of Treatment (Simple or Complex) and Trial order (1^{st} or 2^{nd} order of presentation) with song response to playback (PC2). Results from Tukey method for post hoc comparisons among the least-squares means treatments. Marginal to significance italicised.

Treatment Pairwise comparison	β	SE	df	t	P
1^{st} order, Complex – 2^{nd} order, Complex	-0.26	0.43	30.63	-0.61	0.92
1^{st} order, Complex – 1^{st} order, Simple	-0.48	0.45	27.69	-1.04	0.72
1^{st} order, Complex – 2^{nd} order, Simple	0.59	0.46	43.54	1.28	0.57
2^{nd} order, Complex – 1^{st} order, Simple	-0.21	0.39	40.41	-0.53	0.94
2^{nd} order, Complex – 2^{nd} order Simple	0.86	0.40	40.23	2.12	0.16
1^{st} order, Simple – 2^{nd} order Simple	1.07	0.43	34.28	2.47	*0.08*

Chapter 6

General discussion

General Discussion

My research contributes to an improved understanding of the behaviour and breeding biology of the lovely fairy-wren (LFW) as well as of the form and function of plumage colouration and song in females and males of this species. More generally, it shows how ecological and social differences and similarities between sexes may shape ornamentation. Below I discuss my main findings and suggest directions for future research.

Summary and key findings

In **Chapter 1**, I reviewed the theoretical framework and discussed the different hypotheses that may explain the evolution of female ornamentation – genetic correlation, sexual selection, and social selection - and reasoned that the genus *Malurus,* and lovely fairy-wrens in particular, provide an ideal model to test the different hypotheses proposed.

In **Chapter 2**, I provided a detailed description of the ecology, behaviour, and breeding biology of the LFW, based on studies of a colour-banded population over five years. I found that the LFW has characteristics consistent with other tropical birds, with groups maintaining territories and breeding intermittently throughout the year, small clutch size, and long juvenile dependence. Adults maintained long-term pair bonds, with high adult breeder survival, and similar male and female parental roles. LFW bred cooperatively, but group sizes were small, and this could be a consequence of low productivity, driven by high nest predation. I also show that LFW are sexually size-dimorphic, with males being slightly larger than females. Surprisingly, unlike other Australian fairy-wrens, males maintained their bright colour adult plumage year-round after initial acquisition (Table 1 in **Chapter 2**). Coupled with the unusually colourful plumage of females, this suggests there may be similarities in selection on plumage colour in males and females of this species. Overall, in addition to serving as a foundation for the next chapters, this study contributes to our understanding of the biology and natural history of the LFW, the diversity of the fairy-wrens, and tropical species in general.

In **Chapter 3,** I studied whether plumage colour is a signal, and if it functions in competitive contexts for females and males. I used a mirror image stimulation test to first assess the relationship between male and female natural plumage colour and agonistic behaviour. I then experimentally manipulated plumage colour and measured individual responses to their mirror image reflection. In the first experiment, females and males were more aggressive towards naturally less colourful reflections of the cheek patch in the mirror. This indicates that individuals may use plumage colour as a signal of social status and are more likely to attack a less colourful if this is perceived as a weaker rival. In the second experiment, when I manipulated plumage colour, both females and males responded more aggressively to experimentally increased cheek colour reflection in the mirror, which might be indicative of social punishment in the form of aggression towards ‘dishonest’ more colourful individuals. Experiments 1 and 2 provided somewhat counterintuitive results, but together suggest that plumage colour may not signal individual aggression *per se*, but rather function as a signal in aggressive contexts in both sexes, and that signal reliability may be maintained by social interactions where individuals police and punish dishonest signals. To my knowledge, this is the first study using the mirror image stimulation test to understand the function of plumage colour, and this method has promise for future studies, particularly to test short-range visual signals.

Since LFW breed year-round, unlike most Australian fairy-wrens (**Chapter 2**), it is possible that plumage colour is also used in intersexual interactions such as mate choice. In **Chapter 4,** I assessed whether plumage ornamentation in male and female LFW is sexually selected. I first examined the relation between plumage colour and measures of quality, reproductive success, and survival to understand whether plumage colour might act as honest signal or has potential survival costs. I found that female and male plumage colour was uncorrelated with measures of individual quality, survival, and reproductive success. I also examined the relationship between plumage traits and patterns of pairing and paternity. Females and their social males paired assortatively by plumage colour and parental care. This mating patterns suggest that plumage colour is favoured by mutual sexual selection. However, a similar pattern could arise if resource abundance (territory quality) relates to both plumage colour and provisioning rates, particularly if these are resource-dependent. Behavioural observations and experiments are needed to test these ideas (see *Future directions*).

Microsatellite analyses of paternity indicated that 53% of offspring were sired by extra-pair males, and higher rates of extra-pair paternity occurred where density of breeding pairs was high. Females with helpers also tended to have more extra-pair offspring. Female and male plumage colour did not predict the proportion of extra-pair offspring in their own nest, but less colourful males obtained higher extra-pair and total paternity. Males chosen by females as extra-pair mates were also bigger than the female's social partner. Overall, these results indicate that plumage colour plays different roles in male and female mating strategies, and emphasises the importance of considering both sexes in studies of sexual selection. Females (but not males) may exert some mate choice based on colour and/or less colourful males have intrinsic behavioural differences from more colourful ones – such as willingness to foray for extra-pair mates. The exact causes of the relation between plumage colours and paternity remains unknown, and I recommend that this should be explored further (see *Future directions*).

Recent research suggests female song is common and phylogenetically widespread, but there are still only a few species for which song similarities and differences between the sexes have been described. This is important in order to understand functional and evolutionary differences between female and male traits. In **Chapter 5**, I investigated the function of female and male song in the Lovely fairy-wren. I analysed song structure and compared song rates across seasons, breeding stages and when partners were together or apart. Females and males had similar song metrics and natural song rates. Pairs sang more during non-breeding periods than during breeding periods. Within each breeding stage, males sang more than females when females were incubating. During incubation the female is more often apart from her partner, since females take sole care of incubation (**Chapter 2**). When I quantified what proportion of songs individuals sang when together or apart, both females and males sang more of their songs when they were apart from their partner during nest building and incubation. Therefore, it is possible that individuals use song to maintain contact with their partner in their dense habitat when they are apart from each other. I also tested individual responses to simulated territorial intrusion, using playbacks of females and males with songs varying in complexity (more or less complex songs). Females and males responded to simulated intrusion with coordinated and similar responses. Male song playbacks elicited stronger responses than female song playbacks from both sexes, as did simple playback songs after they were exposed to complex songs, during both breeding and non-breeding periods. Male intruders may represent a greater threat than females, and simpler songs are possibly perceived as a “weaker” intruder; when hearing a less threatening song after a complex one, pairs may be more encouraged to attack. Overall, observations and experiments indicate that female and male songs function primarily in within-pair communication and joint defence of resources such as territories.

Function of female (and male) ornamentation

Taken together, the findings of this thesis suggest that both song and plumage colouration in females are important signalling components in different contexts, and that, as in males, selection favours female expression of traits. Plumage colour in females and males does not seem to incur high survival and reproductive costs, nor does it appear to convey individual quality (**Chapter 2 and 4**). It is possible that plumage colour does not function as a condition-dependent index (Guilford & Dawkins, 1995) but instead its honesty is maintained by social costs (**Chapter 3**). Potential fitness costs for song were not quantified and further detailed studies would be required to understand this and mechanisms for signal honesty (*see Future directions*).

Fairy-wrens are variable in plumage dichromatism, and previous work has shown patterns of directional plumage colour evolution on both female and male plumage (Friedman & Remeš, 2015; Johnson et al., 2013). In LFW, because females and males are highly colourful year-round (**Chapter 2**), adult survival is high (**Chapter 2**), and colour differences between individuals are unrelated to survival (**Chapter 4),** plumage might not be under strong natural selection**.** Across fairy-wrens, species with more colourfully plumaged females occupy closed habitats, while dull-plumaged females rend to occupy more open habitat, and latitude is correlated with vegetation density (Medina et al., 2017). This indicates that predation can be a selective pressure for plumage conspicuousness. If predation-risk is a potential cost for bright colours, this could explain why LFW females are colourful, and the variation in plumage colours in fairy-wrens in general. To address this, I collaborated with Kristal Cain and other researchers (Cain et al., 2019), to determine the relationship between bright and dull plumages and predation-risk by measuring attack rates on fairy-wren 3D-printed and hand-painted models that were placed in different fairy-wren habitats around Australia. We found that predation-risk was higher in southern and open habitats, but dull models were attacked as often as conspicuous models, and in some sites more often. The results show that colour in fairy-wrens does not increase the predation risk, and that being bright may interact with other factors in driving the evolution of colourful plumages (Cain et al., 2019).

Thus, ecological factors might help shape ornament expression but are unlikely to be the sole determinants. The extended breeding period, low territory turnover, and high female dispersal (**Chapter 2**) suggest that competition for territories or reproductive resources is intense. Plumage colours in this species serve as signals of status in competitive contexts (**Chapter 3**) which may assist in year-round territorial defence. Fairy-wren species in northern latitudes maintain territories year-round, while those living in southern latitudes do not defend their territories during non-breeding season (Rowley & Russell, 1997), so females and males may benefit from retaining colourful plumages for territorial defence.

Song structure was similar rather than sexually distinctive between sexes, individuals sang at similar but lower rates during breeding stages, and responses to simulated intruders were similar outside the breeding period. These findings suggest that mate choice is not the primary function of song elaboration. By contrast, competition seems to be a key selective pressure shaping song, considering that individuals sang year-round and more during non-breeding rather than during a specific breeding period, and the sexes responded with similar intensities and in a coordinated way to intrusions during breeding and non-breeding periods (**Chapter 5**).

In the tropics, habitat may be saturated and competition for partners or territories can be intense (Slater & Mann, 2004; Stutchbury & Morton, 2001). The low adult mortality and consequently low territory turnover observed in this species suggests there is high territorial pressure. Females and males may share mutual benefits in defending territories, and joint territorial defence is possibly more effective at deterring intruders.

Overall, visual and acoustic ornaments were important to both females and males and are used as signals to mediate social interactions. Plumage colour in females and males appears to convey similar or different information depending on the context: in males, it may follow the classical pattern of sexual selection, functioning in mate choice and male-male competition (**Chapter 3** and **4**), while in females, plumage colours do not seem to be influenced by male choice, but function in same-sex competitive contexts (**Chapter 3)**. These different contexts might explain the patterns of dichromatism, rather than monochromatism, in this species. Song traits, on the other end, seems to have a convergent function in both sexes, as females and males have similar song structure and used song year-round in identical contexts for within-pair communication and joint territorial defence (**Chapter 5**).

A multicomponent signalling system may benefit Lovely fairy-wrens. Song might work better in long distance communication, especially effective on this species that inhabit large territories in dense habitats. Their bright colours might operate for short distance communication, where light can be less effective due to foliage density. In natural settings, long-range acoustic signals usually precede short-range visual signals in escalating interactions in birds (Bradbury & Vehrencamp, 1998), and the mirror image stimulation tests confirmed that LFW used visual signals more than vocal signals in short-range interactions. If selection favours more competitive individuals, different signals that mediate competition for resources can be important to effectively reduce consequences of fighting. The exact relation between colour and song traits is unknown, and I recommend that this should be addressed in the future (see *Future directions*).

My findings urge for parallel studies on other species, to reconcile some of the results of my research and understand how broadly they apply. Insights from a close relative to the LFW species such as the *Malurus assimilis* (*dulcis* and *rogersi*) (McLean et al., 2017), which have somewhat colourfully plumaged females, would be an ideal system to further investigate the significance of female ornaments. I am collaborating with Michelle Hall and my lab on a detailed investigation of the tropical *M. a. dulcis*, addressing similar questions to the ones addressed in my thesis, in order to understand the function of female signal traits. Adding more empirical studies focusing on taxonomically similar species will allow a comparison of factors other than phylogeny driving interspecific variation in female signalling, and improve our understanding of the processes of female ornament evolution.

Unresolved questions and future directions

Below, I briefly outline new questions that have arisen from this research, and suggest areas of opportunity for future research.

Why do more colourful males sire fewer extra-pair young?

In **Chapter 4** I found that less colourful males sired more extra-pair young, which suggests that sexual selection is acting on the plumage colour of male lovely fairy-wrens, though the mechanisms are unclear. Brighter plumage was expected to relate with individual quality and used by females during mate choice. If females display a preference for less colourful males, it is not clear what females gain by such choice. Understanding whether plumage is costly to produce or if high colourfulness signals quality would help explain this relation between paternity success and plumage colour (see below).

Does plumage colour and song signal individual quality?

In **Chapter 4**, we did not find any relation between plumage colour and measures of quality (parental care, body size and body condition). If this is the case, plumage colour might be considered a conventional signal, and these do not have obvious costs in production, and may not be associated with condition. In fact, from C**hapter 3** it is suggested that signal reliability might be maintained by social interactions.

It would be important to complement the measures of possible quality and condition taken in this thesis and extend it in relation to song traits. For this, I already collected other measures such as faecal samples for endoparasite analysis, wing photos for feather ectoparasite presence, blood smears for measures of stress and immune capacity, to relate to colour measurements and song recordings. I expect that this will give a more complete picture of whether plumage colour and song relate with measures of individual quality, and if they do, whether there are overlapping or distinct signalling contexts in both females and males.

Does plumage colour function during territorial intrusions?

Female and male plumage colour signals competitive ability in same-sex aggressive context (**Chapter 3**). However, the fact that experiment 1 and experiment 2 results showed opposite patterns indicates that more research is needed to establish the precise nature of the link between plumage colour and aggression. Particularly, future experiments should find a treatment that effectively reduces the colour to understand whether less colourful birds are still more aggressive (as in experiment 1). It is also unclear in which context plumage colouration functions, specifically, how individuals react to differences in plumage colour in response to a territorial intrusion. For this purpose, I already conducted an experiment to test whether female and male response towards a decoy (3D-printed model) was related to the plumage colours of both intruding (decoy) and resident (their own colour) pairs. For this I simultaneously presented two models of the same sex to resident pairs; one model had reduced and the other had increased colour. By experimenting with female and male models I can compare and test whether colour and behavioural response is sex-specific, i.e. females and males respond differently to females and male intruders.

How are plumage traits and song related to access to resources? And how important are resources versus predators in determining territory quality?

The expression of competitive traits might be important, for both females and males, when resources are critical. To address whether and what ecological drivers affect plumage colour and song, future studies should address how ornamental traits relate with resources such as territories, and how territories affect predation-risk and reproductive success. In particular, what makes a territory of good quality? What are the costs and benefits of high-quality territory? And do more ornamented individuals defend higher quality territories? These questions are currently being addressed with two measures of variation in territory quality: (i) resource abundance: I collected insects from each territory as a proxy for food abundance and (ii) predation-risk: I conducted an experiment with 3D printed models to assess differences in predation risk between territories. Additionally, using data collected for this thesis, such as territory size (**Chapter 2**), reproductive success of the pairs that occupy these territories

(**Chapter 4**), and their plumage colour (**Chapter 3 and 4**) and song (**Chapter 3 and 5**), I plan to understand the relation between territory quality and ornamental traits. I expect that this work will inform whether female (and male) plumages and song are related with reproductive and non-reproductive resources, to infer other functions and selective pressures driving the expression of ornaments.

How does male and female song relate to ecological and mating systems across different species?

To understand the evolution of female ornamentation it is important to focus on the different factors that are shaping female ornaments in different species. I am contributing to a collaborative study with Karan Odom (Cornell Laboratory of Ornithology), to evaluate song elaboration and possible correlation in both sexes for fairy-wren species. Preliminary results have found little difference between female and male song structure, but songs of both sexes were elaborate: both female and male songs contained a diversity of note structures covering broad frequency ranges. Song elaboration in both females and males was negatively correlated to latitude and the number of subordinates within breeding groups. Therefore, social and environmental pressures may play a role in the evolution of song elaboration in both sexes in fairy-wrens.

Concluding remarks

This thesis provides insight into the form and function of both female and male plumage colours and song in a tropical species. First, it shows that song and plumage colour is a signal in females, suggesting that ornaments in this species are not just a correlated genetic by-product of traits in males, and that selection favours female (and male) expression of traits. Second, it highlights that song has convergent functions in both sexes for territorial defence and communication within-pair. Third, the information conveyed by plumage colouration seems to be context-dependent in relation to the sex of the bearer, which might explain dichromatism in this species. The fact that females and males sing and have bright colours year-round in parallel with their territorial and breeding behaviour, suggests that individuals use their traits to maintain sexual and non-sexual resources. Further work is required to determine the importance of these resources in shaping female and male ornaments.

This thesis project is one of the few studies to describe and directly compare female and male signal function, which I believe is important and necessary for a realistic understanding of ornament evolution. It also gives rise to novel questions in several fields such behavioural and ornament signalling, mating systems, and provides empirical data for future comparative analyses of plumage colour and song in the fairy-wrens and other taxonomical groups.

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