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EVOLUTION AND SOCIALITY IN FAIRY-WRENS (AVES: MALURIDAE)

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ALLISON ELAINE JOHNSON

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CHAPTER 1: EVOLUTION AND SOCIALITY IN FAIRY-WRENS (AVES: MALURIDAE)

INTRODUCTION

The evolution and diversity of social behavior have long been a focus of research for behavioral ecologists and evolutionary biologists. From brief interactions between individuals of solitary species for mating purposes, to highly complex associations between individuals of cooperative or colonial species, understanding sociality has been central to the studies of sexual selection, evolution, and adaptation (Alexander 1974, Emlen and Oring 1977, Akçay et al. 2015, Rehan and Toth 2015). The animal taxa most useful for examining theories of sociality are those that exhibit a diversity of sociality and ecology. The Maluridae comprise a family of highly social birds native to Australia and New Guinea that have been the focus of study for many aspects of sociality, and have helped to further develop our understanding of cooperative breeding, brood parasitism, sperm competition and even social song transfer (Pruett-Jones and Lewis 1990, Mulder et al. 1994, Rowley and Russell 1997, Langmore et al. 2003, Rowe and Pruett-Jones 2006, Greig and Webster 2013, Greig et al. 2013).

The first species of bird documented to exhibit cooperative breeding behavior was a fairy-wren (Boland and Cockburn 2002), and since then, every species in the genus *Malurus*, including the superb (*M. cyaneus*), splendid (*M. splendens*), red-backed (*M. melanocephalus*), red-winged (M. elegans), purple-crowned (*M. coronatus*), and white-winged fairy-wrens (*M. leucopterus*) (Dunn and Cockburn 1996, Driskell et al. 2002, Karubian 2002, Webster et al. 2004, Kingma et al. 2009, Brouwer et al. 2011) as well as one species of emu-wren, the southern

emu-wren (*Stipiturus malachurus*, Maguire and Mulder 2008) and one species of grasswren, the striated grasswren (*Amytornis striatus*, Karubian 2001), have been documented as cooperative breeders. While these species serve as models for cooperative breeding, they also have a number of atypical characteristics that make them ideal for the study of social systems. Most notably, these species have extremely high rates of extra-pair paternity, and exhibit variation in the identity (sex) or and the role that auxiliary individuals take in the social group (Mulder et al. 1994, Webster et al. 2004). The percentage of extra-pair young across species has been found to range anywhere from 4.4% to 76% (Mulder et al. 1994, Webster et al. 2004, Maguire and Mulder 2008, Kingma et al. 2009). In addition, helper number and sex varies both within species across habitats and among species (Russell and Rowley 1993, Van Bael and Pruett-Jones 2000). Of additional interest is their extreme geographic range, which spans portions of arid south Australia up to tropical northern Australia and New Guinea. This geographic variation and geographic barriers within Australia have been implicated both in past and ongoing speciation (Mclean et al. 2012, Joseph et al. 2013). Together these factors lend the family to studies of trait evolution and the costs and benefits of sociality (Rowley and Russell 2007).

My work aims to better understand the evolution of and sociality within fairy-wrens (Maluridae) through comparative analyses and, specifically, by characterizing the behavior of one of the more extreme cooperative members of this family, the variegated fairy-wren (*M. lamberti*). *Chapter 2: Different modes of evolution in males and females generate dichromatism in fairy-wrens*, was conducted in collaboration with Drs. Jordan Price and Stephen Pruett-Jones. We sought to explain whether evolution in male or female plumage color influences the degree of plumage difference between the sexes, and how this dichromatism relates to latitude. After scoring plumage for males and females for all species in the Maluridae, we reconstructed ancestral plumages states

for both sexes separately, correlated plumage distance versus molecular sequence divergence, and finally fit models of evolution to determine if plumage changes in males and females corresponded to different evolutionary regimes. We found that while males have accumulated changes in plumage steadily over time, females changed rapidly in some lineages but little to none in others. These patterns in males and females fit different modes of evolution, with plumage evolution in males best fitting a Brownian motion model, which describes a random walk or randomly fluctuating selection. In contrast, the pattern in females best fit a multi-optimum Ornstein Uhlenbeck model with adaptive peaks corresponding to geographic distribution in Australia or New Guinea, a model that suggests natural selection. Plumage dichromatism between males and females correlated significantly with latitude, with males and females looking more divergent the further south they ranged. While more generally plumage evolution is likely the result of change occurring across both sexes, we hypothesize that change in the Maluridae is driven largely by environmental or social selection on female plumage (Johnson et al. 2013).

In *Chapter 3: Heterospecific sociality mediated by song discrimination in fairy-wrens*, I document an example of heterospecific sociality between splendid and variegated fairy-wrens and experimentally test discrimination between co-resident, neighbor and foreign heterospecifics by song. Frequently these species are found to occur on coincident territories, and in these cases appear to jointly defend the territory from intruders of both species. I examined foraging and time budgets in both species when alone and when associating with their co-resident to determine whether associating with the other species results in behavioral changes. I also monitored breeding on shared and solitary territories to determine if sharing a territory improves fledging success or likelihood of re-nesting following a nest failure. I tested discrimination between co-residents and neighbor or foreign heterospecifics (birds they had not heard before)

for both species to determine if tolerance of heterospecifics was limited to the co-resident. Finally, I tested neighbor-stranger discrimination within species to determine if discrimination between neighboring and foreign individuals occurs. Recognition studies have not been carried out in members of this family before and it was critical to document any pattern within species in order to understand the patterns between species. I found that variegated fairy-wrens decreased the amount of time they spent traveling, increased the amount of time spent foraging, and decreased vigilance when associating with co-residents. More variegated fairy-wren groups fledged their first nesting attempt and more variegated fairy-wren groups re-nested following initial nest failure on shared territories than on solitary territories. While splendid fairy-wrens did not exhibit these changes, both species responded less strongly to songs of their heterospecific overlap than to either neighboring or foreign heterospecifics. However, neither species discriminated between neighboring or foreign heterospecific songs. Within species, individuals did not discriminate between neighboring or foreign songs. While it is known that heterospecific associations can alter species success and behavior, these patterns are generally attributed to general associations rather than associations between specific individuals (Seppänen et al. 2007, Goodale et al. 2010). These data suggest that heterospecific sociality between specific groups may similarly influence behavior. I hypothesize that these birds form social groups that extend beyond the species and that these associations are more important for variegated fairy-wrens, in which large group size strongly influences the likelihood that a group with fledge offspring (see Chapter 4). These associations may be "pay-to-stay" (Gaston 1978, Kokko et al. 2002), in which both species defend the territory from heterospecifics as well as conspecifics in order to gain benefits from the other species, or to be allowed to remain on the territory.

In *Chapter 4: Helping behavior and promiscuity in the variegated fairy-wren: sex specific effects of auxiliary members*, I characterize the social behavior of one of the most social species of fairy-wren. Between 2012 and 2015 I followed variegated fairy-wren social groups quantifying group size, composition, nesting success, and chick weight and egg volume across different group sizes. I also monitored provisioning behavior at nests by dominant and auxiliary individuals to determine how total provisioning rate and provisioning behavior of breeding individuals are influenced by the presence of male or female auxiliary group members. Finally, I quantified extra-pair paternity rates to determine whether the presence of male or female auxiliaries altered the female's likelihood to engage in extra-pair matings. I found that fledging success increased with the presence of male but not female auxiliaries. Egg volume positively increased with number of male, but not female auxiliaries, while chick weight at 6 days old increased with the number of female but not male auxiliaries. In general dominant female provisioning rate was best predicted by the feeding rate of the dominant male, however, male provisioning rate decreased as the number of male auxiliaries increased. Finally, extra-pair paternity rate *decreased* as the number of male auxiliaries increased, the reverse of the pattern found in other fairy-wren species (Mulder et al. 1994, Webster et al. 2004). This illustrates the strong role that not only group size, but group composition, plays on nesting success and behavior. In particular, decreased rates of extra-pair paternity in the setting of increased number of male auxiliaries may suggest that variegated fairy-wrens are a more typical cooperative breeder than other fairy-wrens, and may exhibit a trend towards the reproductive monogamy expected in cooperatively breeding species as group size increases (Cornwallis et al. 2010).

CONCLUSIONS

Fairy-wrens continue to be an ideal system to study evolutionary pressures and the evolution of sociality. After we published *Chapter 2: Different modes of evolution in males and females generate dichromatism in fairy-wrens*, another group of researchers conducted a similar study of plumage evolution in fairy-wrens, examining plumage with reflectance spectra rather than visually scored patches (Friedman and Remeš 2015). They found that males are evolving more rapidly than females, however, they also suggested that this was the result of differing pressures on males and females (Friedman and Remeš 2015). The question still remains, why are some females bright, and why is there a correlation with latitude? As species living in drier southern latitudes become nomadic during the non-breeding season, while species living in wetter northern latitudes may maintain territories year round, females in northern latitudes may need to assist in territory defense and may retain bright plumage as the result of social selection (West-Eberhard 1983, Johnson et al. 2013, Friedman and Remeš 2015). Females in southern latitudes with more open, drier habitat and shorter breeding seasons may be under selection to maintain crypsis to avoid predation (Hamilton 1961). The extreme variation in habitat and social behavior in Maluridae lends this family to further investigation of these questions (Karubian 2013). Indeed, work currently being carried out Kristal Cain at Australian National University, is seeking to determine the cost associated with bright plumage in females.

My work on heterospecific sociality between splendid and variegated fairy-wrens suggests there may be implications for reproduction and behavior by forming social groups across species. However, more work needs to be done to understand whether this association is truly adaptive, or if it is the result of an inability to exclude a heterospecific group, thereby functionally making the best of a bad situation. Habituation experiments, in which birds living on

non-overlapping territories are presented with song stimuli of heterospecifics, could be performed to determine if a lack of response to co-residents is simply a result of habituation. Presentation of foreign heterospecific songs to birds on non-overlapping territories could be performed to determine if aggressive responses to heterospecifics are normal or if they only occur when sharing a territory. It is possible that associating with a heterospecific increases the probability of detecting predators and defending nests. Presentations of simulated predators at the nest site of groups with and without co-residents could be performed to answer whether this may be an adaptive advantage to co-residency. Finally, during our collection of songs from both species, previously hypothesized cases of social transmission of song type across species were noted. Splendid and variegated songs, while containing similar elements, sound quite different, and we documented a few cases of splendid fairy-wrens singing Type II suffixes that sound like variegated fairy-wrens, and vice versa. Forming heterospecific social groups may have implications for song evolution, with species either trying to sound more similar to maintain group cohesiveness, or alternatively sounding more dissimilar to prevent misidentification of species. While there is extreme variation in range of Maluridae species, there is also much overlap between species, and other fairy-wren species may lend themselves to more investigation of this kind. I plan to continue to work in this system to answer some of these questions.

The fairy-wrens were initially studied to understand cooperative breeding in birds. However, their high rates of extra-pair paternity, strong sexual selection, and extreme variation in group size and number have made them somewhat distinct from typical cooperative breeders who are expected to be reproductively monogamous (Cockburn 2006, Rowe and Pruett-Jones 2006, Cornwallis et al. 2010). That the variegated fairy-wren seems more dependent on the presence of helpers to successfully fledge young and have lower rates of extra-pair paternity

suggests this species is more typical of other cooperatively breeding birds rather than other fairy-wrens. It also suggests that the variation in average group size across the Maluridae may represent evolution towards or away from cooperative breeding behavior, with some populations possibly loosing cooperative breeding while others, like the variegated fairy-wren, may becoming obligate cooperative breeders. Extreme differences in ecology both between and within species, paired with varying degrees of cooperative breeding, lend this family to a closer examination of the evolutionary pressures underlying cooperative breeding. Though extensive research has been performed on cooperative breeding in birds over the last 50 years (Brown 1987), inconsistencies between cooperative breeding theory and known cases of cooperative breeding species remain. Our lab and other researchers are currently collaborating with Lyanne Brouwer at the Australian National University to complete a comparative analysis of extra-pair paternity across the Maluridae family. Additionally, because of the interesting patterns of social behavior and extra-pair paternity found in the variegated fairy-wren, this species would be ideal for a replication of the study completed by Pruett-Jones and Lewis (1999). That study experimentally tested the habitat saturation theory of cooperative breeding, and suggested that birds delay their dispersal when there are no available territories or breeding vacancies. The importance of this idea may vary when helping behavior at the nest is necessary to the breeding success of the dominant pair. Studying this system in more depth may help elucidate the underlying drives of cooperative breeding behavior.

My work, and the questions raised by it, supports the continued study of fairy-wrens and their relatives to understand sociality. More comparative studies across members of this species may help to explain evolutionary drivers of dimorphic trait evolution, cooperative breeding, and sociality beyond the species.

CHAPTER 2: DIFFERENT MODES OF EVOLUTION IN MALES AND FEMALES GENERATE DICHROMATISM IN FAIRY-WRENS (MALURIDAE)[1]

ABSTRACT

Sexual dichromatism in birds is often attributed to selection for elaboration in males. Yet evolutionary changes in either sex can result in plumage differences between them, and such changes can result in either gains or losses of dimorphism. We reconstructed the evolution of plumage colors in both males and females of species in Maluridae, a family comprising the fairy-wrens (*Malurus, Clytomias, Sipodotus*), emu-wrens (*Stipiturus*), and grasswrens (*Amytornis*). Our results show that, across species, males and females differ in their patterns of color evolution. Male plumage has diverged at relatively steady rates, whereas female coloration has changed dramatically in some lineages and little in others. Accordingly, in comparisons against evolutionary models, plumage changes in males best fit a Brownian motion (BM) model, whereas plumage changes in females fit an Ornstein Uhlenbeck (OU) multi-optimum model, with different adaptive peaks corresponding to distributions in either Australia or New Guinea. Levels of dichromatism were significantly associated with latitude, with greater dichromatism in more southerly taxa. Our results suggest that current patterns of plumage diversity in fairy-wrens are a product of evolutionary changes in both sexes, driven in part by environmental differences across the distribution of the family.

[1] This manuscript is reprinted with permission: Johnson, A. E., Price, J. J. and Pruett-Jones, S. (2013). *Ecol. Evol.* **3**, 3030-3046.

INTRODUCTION

The evolution and diversity of coloration in birds has long interested evolutionary biologists (Darwin 1987, Cronin 1991, Andersson 1994). Both sexual dichromatism and monochromatism are common in birds, and at least 150 independent transitions between these states have occurred within the passerines alone (Price and Birch 1996, but see Eaton 2005). This suggests that plumage is subject to many different and fluctuating selection pressures. Dichromatism results when selection differs between the sexes, either for increased or decreased ornamentation in either or both males and females (Cunningham and Birkhead 1998, Kimball and Ligon 1999, Amundsen 2000, Badyaev and Hill 2003). In fact, dimorphism in any trait can result from changes in either sex away from a shared pattern (Irwin 1994, Burns 1998, Wiens 2001, Hofmann et al. 2008, Friedman et al. 2009, Price et al. 2009).

Although ecological factors (e.g., breeding latitude, predation) are known to influence the evolution of dichromatism (Bennett and Owens 2002, Badyaev and Hill 2003), the most widely accepted paradigm involves sexual selection for elaboration of plumage in males (Andersson 1994, Amundsen 2000). The assumption implicit in this hypothesis is that species with elaborate or bright males and relatively dull females evolved from an ancestral condition in which both males and females were dull in plumage. Nevertheless, plumage in females can show considerable variation among taxa, and female plumage coloration is often under strong selection (Björklund 1991, Irwin 1994, Martin and Badyaev 1996, Burns 1998, Cunningham and Birkhead 1998, Amundsen 2000, Hofmann et al. 2008, Friedman et al. 2009). In at least one genus of birds, the New World orioles (*Icterus*), evidence suggests that sexual dichromatism has resulted from the repeated loss of bright plumage in females rather than gain of bright plumage in males (Hoffman et al. 2008, Friedman et al. 2009). Even in cases of sexual monochromatism in which

both males and females are brightly colored, such patterns can result from mutual selection by males and females (Jones and Hunter 1993, 1999), social selection on females to retain bright plumage or ornamentation (Irwin 1994, Amundsen 2000), or even as a genetically correlated response to selection on males (Price and Whalen 2009).

In this study we examine plumage evolution in the Maluridae, a relatively small (25 species) but diverse family of birds, comprising the fairy-wrens (*Malurus, Clytomias, Sipodotus*), grasswrens (*Amytornis*), and emu-wrens (*Stiptiturus*). The Maluridae are endemic to Australia and New Guinea, and they evolved in Australasia as part of the ancient songbird radiation (Sibley and Ahlquist 1985, 1990; Christidis and Schodde 1997, Rowley and Russell 1997). Species occur in a wide range of habitats, from primary rainforest in New Guinea to desert grasslands in Australia, and in any geographical area as few as one species or as many as five species may occur. Plumage varies considerably both across and within species (Rowley and Russell 1997, Driskell et al. 2002, 2010, Karubian 2002), and levels of dichromatism differ across species as well. Dichromatism can also differ across populations within one species (e.g., *M. alboscapulatus*). Sexual selection is known to be important in many species of fairy-wrens (Webster et al. 2007), but the extent to which sexual selection influences coloration is presently unknown.

Our focus in this study was on the 21 malurid taxa included in the recent phylogeny by Driskell et al. (2011). Our objectives were to compare the evolution of plumage colors in males and females and to examine how these evolutionary changes have contributed to the evolution of sexual dichromatism in this group.

METHODS

Study system

Our study used the molecular phylogeny and DNA sequence data published by Driskell et al. (2011), and both that study and ours follow the taxonomic nomenclature of Dickinson (2003) and Christidis and Boles (2008). The phylogeny of Driskell et al. (2011) supports the general classification of the Australian fairy-wrens (*Malurus*) into several commonly recognized 'coloration groups' (Christidis and Schodde 1997): the "blue group," consisting of *Malurus cyaneus* and *M. splendens*, the "bi-colored group," consisting of *M. alboscapulatus*, *M. melanocephalus* and *M. leucopterus*, and the "chestnut-shouldered group," consisting of *M. elegans, M. lamberti, M. pulcherrimus* and *M. amabilis*, with *M. grayi*, *M. cyanocephalus*, and *M. coronatus* forming additional distinct color groups (Rowley and Russell 2007).

Plumage scoring

Our methods for choosing and scoring plumage characters followed methods used by Omland and Lanyon (2000) and Price and Whalen (2009). Our study included 22 taxa representing 16 species, including all of the Australian fairy-wren taxa (genus *Malurus*), most of the New Guinea fairy-wren taxa (*Malurus, Clytomias* and *Sipodotus*), one emu-wren (*Stipiturus malachurus*), one grasswren (*Amytornis striatus*), and a honeyeater (*Acanthorhynchus tenuirostris*) as an outgroup. The latter two species were included as outgroup taxa in the phylogeny of Driskell et al. (2011).

Plumage patches were scored as discrete characters for adult males and females of each of the 22 taxa. Although spectrometric methods are available for measuring color (e.g., Eaton

2005; Hofmann et al. 2008), our phylogenetic analyses (see below) required that we score color as discrete character states rather than as continuous reflectance values. We first scored plumage colors based on the illustrations in Rowley and Russell (1997). These scores were checked against illustrations in Schodde (1982). We then confirmed or revised these scores using museum skins from the collection at the Field Museum of Natural History (Chicago, IL) for males of 13 species and females of 12 species (57 specimens total). Except for one trait (coloration of the back), the scores we obtained from examination of the study skins were identical to those scores obtained from the illustrations in Schodde (1982) and Rowley and Russell (1997).

In all, we scored 25 color patches (body regions) and the presence/absence of five qualitative traits (30 characters total for both males and females; Appendices 1 and 2). We defined a color patch as a continuous region of the body with a similar coloration. If a body region (e.g., belly) exhibited color variation within it in any taxon, the region was split into two or more distinct patches (e.g., upper belly and lower belly) for all taxa. Colors were scored as either carotenoid (C), blue (B), black (N), dark brown (D), light brown (L), or white (W). As in the studies by Omland and Lanyon (2000) and Price and Whalen (2009), our color scores were discrete categories that each represented a continuous yet narrow range of colors. Scoring color in this way allowed us to identify discontinuous evolutionary changes in our phylogenetic reconstructions of plumage color evolution (Hofmann et al. 2008).

In some cases, different scores may reflect different underlying mechanisms of coloration, though the accuracy of such relationships likely had little impact on our reconstructions of evolutionary change. For example, the various shades of blue exhibited by many fairy-wrens (Rowley and Russell 1997) are all presumably the result of complex feather microstructure (Prum 2006) and so were combined in our analysis into one character state.

Likewise, reds and oranges in birds are often products of carotenoid pigments (McGraw 2006a, but see McGraw 2004), so we combined these colors as well. Blacks, dark browns, and light browns are generally due to melanin pigmentation in birds (McGraw 2006b); however, these colors differed enough consistently across our study taxa that we categorized them as different character states. Qualitative traits (e.g., whether an eye-line was present, or whether iridescence was prominent in the plumage) were scored as either present or absent.

Of the 30 characters (25 body regions and 5 presence/absence traits), 29 varied across taxa in males and 28 varied in females (Appendices 1 and 2). Beak color was invariant in all males, whereas crown spot and eye-line were invariant in females. Across the 30 characters, the mean number of character states scored in males (3.73 ± 0.24) and in females (3.50 ± 0.27) across species did not differ significantly (paired *t*-test; $t_{29} = 0.851$; $P = 0.402$).

We measured dichromatism in each taxon as the percentage of color characters, of the 30 total, that differed between the sexes. While we determined percent dichromatism, we do not quantitatively or qualitatively describe any species as either monomorphic and bright or monomorphic and dull. Any mention of bright or dull species in the results or discussion is based on our perception of these species and typically we use 'bright' when referring to non-brown hues and 'dull' as brown hues.

Reconstruction of ancestral plumage states

We reconstructed ancestral states for male and female plumage characters separately on the molecular phylogeny of Driskell et al. (2011) using unordered parsimony in MacClade 4.08 (Maddison and Maddison 2005). Other methods of character reconstruction are available, such as Maximum Likelihood and Bayesian methods; however, our goal in this analysis was to identify

statistically discontinuous evolutionary changes in each sex rather than the probability of particular ancestral states on the phylogeny. For each sex, we used the Trace All Changes function in MacClade to count the number of unambiguous (i.e., unequivocal) changes on each branch of the tree. This method calculated the minimum number of changes on a branch by ignoring ambiguous cases in which ancestral changes were dependent on multiple, equally parsimonious states at a corresponding node. We included one of the outgroup taxa used by Driskell et al. (2011), *Amytornis striatus*, in our analysis based on evidence that it is the sister taxon to the rest of the Maluridae (Lee et al. 2012). However, we did not include the non-malurid outgroup taxon, *Acanthorhynchus tenuirostris*.

To explore the effects of alternative phylogenetic hypotheses on our ancestral color reconstructions, we also reconstructed male and female color changes using relationships based on another recent molecular phylogeny of the Maluridae by Lee et al. (2012), which differs from that of Driskell et al. (2011) in the placement of *Malurus coronatus* as sister taxon to all of the Australian *Malurus* rather than as sister taxon to the chestnut-shouldered fairy-wren group (*M. amabilis, M. lamberti, M. pulcherrimus*, and *M. elegans*). We compared the reconstructions using both phylogenies to assess how changes in the placement of *M. coronatus* affected our results.

We assessed the degree to which our plumage data were congruent with phylogeny by calculating the overall consistency index (CI) and overall retention index (RI) separately for males and females using MacClade. For both indices, a score of 1.0 indicates perfect congruence with phylogeny, with no evolutionary convergence or reversals, whereas a score approaching 0.0 indicates high levels of homoplasy. Calculating these scores allowed us to assess whether or not levels of evolutionary convergence in plumage differed between the sexes.

We further investigated evolutionary patterns in male and female coloration by plotting "plumage distance" between all possible pairs of taxa, including *Acanthorhynchus tenuirostris*, as a function of molecular sequence divergence (uncorrected *p* distances), calculated using PAUP* 4.0 (Swofford 2002). We measured plumage distance as the number of plumage characters with different states, and we measured molecular divergence using sequence data from four mitochondrial genes (ND2, ND3, CO1, ATP6) and three nuclear introns (FIB5, LDH, GAPDH; sequences obtained from Driskell et al. 2011). We then calculated a linear regression through the points of each sex and calculated their coefficients of determination (R^2). Although such pair-wise comparisons are not phylogenetically independent (Felsenstein 1985), we nevertheless felt that these relative values provided a useful means for comparing overall patterns of evolutionary change in plumage color between the sexes (also see Price and Whalen 2009).

Testing of evolutionary models

To examine whether plumage colors of males and females have evolved differently, we tested plumage patterns against two evolutionary models. The evolutionary models we considered were Brownian Motion (BM) and single-optimum Ornstein Uhlenbeck (OU) (Butler and King 2004). BM models describe a "random walk," and when applied to evolution can describe either selection randomly fluctuating through time or genetic drift (Felsenstein 1988, Harmon et al. 2010). OU models reflect natural selection toward a trait optimum, genetic drift on a highly constrained character, or genetic drift occurring on a character when stabilizing selection is weak and the adaptive peak is relatively close (Lande 197,; Felsenstein 1988). While other models for character evolution exist (e.g., White Noise, Delta), we chose these two *a*

posteriori based on the results of our analysis of plumage distance versus genetic divergence. Characters were considered unordered and unweighted, i.e., all transitions were considered equally likely. All calculations and simulations were completed in R (R Development Core Team 2012), using the packages "vegan," "geiger," and "OUwie," (Harmon et al. 2009, Beaulieu and O'Meara 2012, Oksanen et al. 2012).

Although fairy-wrens occur in both Australia and New Guinea, the evolutionary history of the group appears to have involved multiple movements and subsequent radiations in different parts of their current distribution (Schodde 1982). It is also the case that Australia and New Guinea have repeatedly been connected by a land bridge, and thus movement from one region to the other may have been relatively easy. It has been hypothesized that the Maluridae first evolved in Australia (leading to today's emu-wren and grasswren clades), that the true fairy-wrens (the *Malurus* [*Chenorhamphus*]–*Sipodotus*–*Clytomyias* clades) evolved in New Guinea, and that fairy-wren (*Malurus*) taxa subsequently radiated on the mainland of Australia followed by a second movement to New Guinea by *M. alboscapulatus* (Schodde 1982) (for examples of each radiation see Fig 1B, A, C & D, and E respectively). The *M. alboscapulatus* complex now includes six recognized subspecies that differ considerably in female plumage colors (Rowley and Russell 1997).

As Australia and New Guinea differ considerably in habitat (Australia being mostly arid and New Guinea being tropical), it seems likely that these differences could influence the evolution of plumage of bird species in each location. With this in mind, we further tested male and female plumage characters against two multi-optimum OU models, treating hypothesized geographic origins of species as adaptive peaks. These models are similar to a single-optimum OU model in that they posit natural selection; however, multi-optimum OU models allow for

multiple adaptive peaks rather that a single adaptive peak. More specifically, our first multi-optimum model had two adaptive peaks corresponding to location (Australia or New Guinea), and the second multi-optimum model had four peaks corresponding to the four distinct distributions outlined above (1. Australian distribution of emu-wrens and grasswrens; 2. New Guinea distribution of the hypothesized basal fairy-wren taxa; 3. Australian species of *Malurus*; and 4. the New Guinea *M. alboscapulatus* complex). For each model we report log-likelihood, Akaike weights, and DeltaAIC (Table 1). Delta AIC values are calculated from AIC values, with the lowest AIC score subtracted from each AIC score to produce the Delta AIC. Thus, the Delta AIC score with the value zero is the best fitting model. If this model is 2 or more points lower than other models it is considered to have substantial empirical support as the best fitting model (Burnham and Anderson 2002). Additional model parameters are available upon request of the authors.

We also reconstructed these four ancestral distribution points as discrete character states on the phylogeny (Driskell et al. 2011). Locations were analyzed as discrete character states as the geographic regions are discrete and separated. This was done using the R package "ape" that employs maximum likelihood methods, similar to those methods employed by Mahler et al. (2010) in reconstructing geography to determine ancestral habitat (Paradis et al. 2004). While there are other reconstruction methods for determining ancestral habitat (e.g., the software Lagrange, Ree and Smith 2008), given that we were using discrete characters, the use of alternative methods would not have altered our results.

Relating dichromatism to latitude

To explore the relationship between latitude and sexual dichromatism, we performed a phylogenetically independent contrasts (PIC) analysis (Felsenstein 1985) using the PDAP:PDTREE module in Mesquite 2.75 (Maddison and Maddison 2011, Midford et al. 2011). This method incorporates phylogenetic relationships into comparisons of continuous variables among taxa to correct for statistical nonindependence due to shared history. We scored latitude (degrees south) as the mean between the upper and lower latitudinal limits of each species' range (Rowley and Russell 1997).

To ensure that the branch lengths of our phylogenetic tree adequately fit the tip data, we initially performed a least squares regression analysis comparing the absolute values of the standardized PIC values versus their standard deviations (see Garland et al. 1992). These regression lines did not differ significantly from zero (dichromatism: $F_{1,18} = 2.522$, $R^2 = 0.123$, $P = 0.130$; latitude: $F_{1,18} = 2.629$, $R^2 = 0.127$, $P = 0.122$), indicating that our tree and model of evolution adequately fit our data. We then tested for a relationship between contrasts of the two traits using linear regression forced through the origin.

RESULTS

Plumage evolution in males and females

Although, in total, more plumage changes were reconstructed in males (146 changes) than in females (135 changes), the mean number of changes in each plumage character on the tree did not differ between the sexes (males: 4.87 ± 0.41; females: 4.5 ± 0.52; paired *t*-test; $t_{29} = 0.73$; $P = 0.471$). Furthermore, male and female plumage characters showed remarkably similar scores for overall CI (males = 0.56; females = 0.56) and overall RI (males = 0.59; females =

0.48), indicating that levels of evolutionary convergence in male plumage and female plumage were similar.

Reconstructing ancestral color changes in each sex on the molecular phylogeny showed that changes in plumage colors have occurred in both sexes throughout the history of the Maluridae (Fig. 2.1; character changes in males/females shown above branches). In some taxa, such as the blue group (*M. cyaneus* and *M. splendens*), nearly all plumage changes have occurred in males and few have occurred in females. In contrast, in other groups, such as the chestnut-shouldered group (*M. elegans*, *M. lamberti*, *M. pulcherrimus* and *M. amabilis*), the greatest number of changes have occurred in females. On 12 branches of the phylogeny (30% of total branches), unambiguous changes occurred in both sexes rather than just one or the other, whereas no unambiguous changes were reconstructed on 9 (22.5%) of the branches.

Some of the most dramatic color changes have occurred in females, which in turn have affected levels of dichromatism. For example, in *M. amabilis*, 15 (50%) plumage characters changed in females while only 1 character changed in males, resulting in much lower levels of dichromatism in this species (27%) than in closely related taxa (>53% in *M. lamberti*, *M. pulcherrimus*, and *M. elegans*; Fig. 2.1). Likewise, 12 (40%) of female plumage characters changed in the ancestor of *M. alboscapulatus* and another 3 (10%) later changed in the subspecies *M. alboscapulatus moretoni*, while no plumage changes occurred at all in males. This subspecies was the only taxon in our study in which males and females exhibited no differences in plumage (Fig. 2.1), and this monochromatism appears to be entirely due to recent, rapid color changes in females.

In plots of plumage distance versus molecular sequence divergence, males and females showed very different evolutionary patterns. These pair-wise comparisons were not corrected for

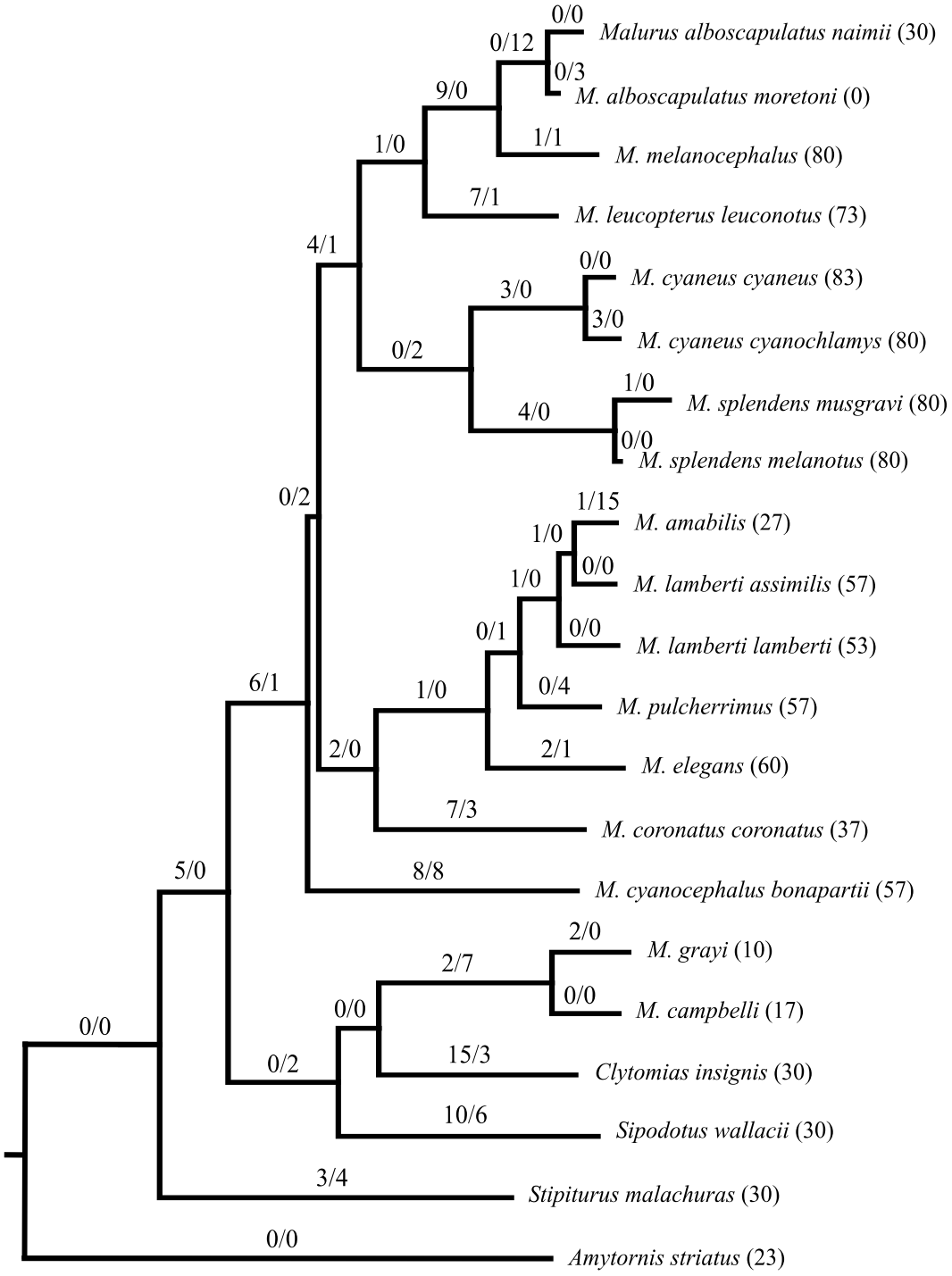

Figure 2.1 Evolutionary changes in plumage characters (indicated for males/female above each branch), measured as the number of unambiguous character state changes in each sex on the molecular phylogeny (from Driskell et al. 2011). Numbers in parentheses beside taxon names are levels of sexual dichromatism measured as the percentage (%) of plumage character differences between males and females within each taxon. Branch lengths on the tree reflect molecular changes.

phylogeny and so should be interpreted with caution, but they nevertheless reveal some striking differences between the sexes. Male plumage differences appear to have accumulated almost linearly as a function of molecular distance between taxa (Fig. 2.2 A; $R^2 = 0.262$; ANOVA: $F_{1,208}$

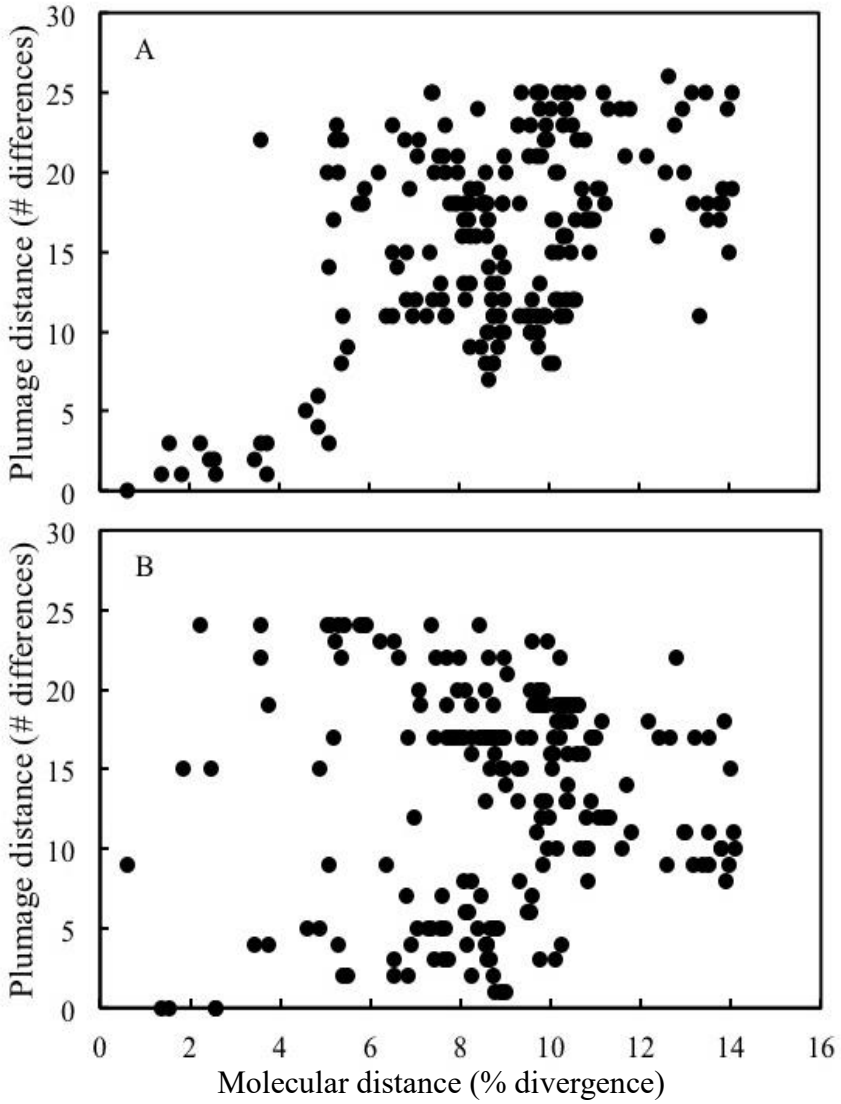

Figure 2.2. Plots showing pair-wise plumage distances between taxa as a function of molecular sequence divergence in (A) males and (B) females. Male plumage differences have accumulated steadily and almost linearly as a function of molecular distance between taxa ($R^2 = 0.262$), whereas female plumage distances show no clear relationship with molecular distance ($R^2 = 0.005$). Molecular divergence values are uncorrected *p* distances based on four mitochondrial and three nuclear regions.

= 73.947; $P < 0.001$), suggesting that male colors have diverged at a relatively constant rate during the evolutionary history of the clade and that male color similarity among taxa is a reasonably accurate indicator of the genetic distance between them. In contrast, female plumage distance shows no clear relationship with molecular distance (Fig. 2.2 B; $R^2 = 0.005$; $F_{1,208} = 0.971$; $P = 0.326$). Female plumage patterns have diverged much more rapidly than male plumage in some cases and much less rapidly in others, suggesting widely varying rates of plumage evolution. Thus, although levels of evolutionary convergence in male plumage and female plumage are not appreciably different, female color patterns provide relatively little information about relationships among taxa.

Fitting the evolutionary models

In line with findings above, plumage color changes in male and female Malurids corresponded to different evolutionary models. The best fitting evolutionary model for male plumage changes was Brownian Motion (BM), and the best fitting model for female changes was a multi-optimum Ornstein Uhlenbeck (OU) model with two adaptive peaks (Table 2.1). For males, the Delta AIC for the BM model was 2 or more points below each of the other three models making this the best fitting model. The three other models (single-peak OU, two-peak OU and four-peak OU) could not be distinguished from one another. For females, the Delta AIC value of the two-peak OU model was approximately 4 points below the next best model (four-peak OU) and more than 20 points below the other two models, making this the best model. Based on this two-peak model, with initial states being either Australia or New Guinea, Australia had a 70.51% likelihood of being the ancestral point of radiation for the Maluridae. For the four-peak model, in which adaptive peaks corresponded to four historical distributions (Schodde

1982), the first category (Australian distribution of emu-wrens and grasswrens) had a 60.39% likelihood of being the ancestral state for the family (Fig. 2.3). Both OU models support the general hypotheses of Schodde (1982) that the family first evolved in Australia and that the genus *Malurus* initially evolved in New Guinea.

Table 2.1. Log-likelihood, Delta AIC, and Akaike weight values from evolutionary models analysis for both male and female Maluridae species.

		Brownian Motion	Single-peak OU	2-peak OU	4-peak OU
Females	Log-likelihood	-1.501	5.443	20.734	22.197
	Delta AIC	38.749	27.563	0	4.322
	Akaike weight	0.000	0.000	0.897	0.103
Male	Log-likelihood	12.093	12.093	12.517	16.360
	Delta AIC	0.000	2.702	4.874	4.435
	Akaike weight	0.687	0.178	0.060	0.075

category (Australian distribution of emu-wrens and grasswrens) had a 60.39% likelihood of being the ancestral state for the family (Fig. 2.3). Both OU models support the general hypotheses of Schodde (1982) that the family first evolved in Australia and that the genus *Malurus* initially evolved in New Guinea.

Our reconstruction of ancestral geography using character states from the four-peak model indicated multiple historical movements of malurid lineages between Australia and New Guinea (Fig. 2.3). This reconstruction suggested that all Australian fairy-wrens originated from New Guinea ancestors and that at least one New Guinea fairy-wren, *M. alboscapulatus*, derived from a subsequent dispersal event back to New Guinea. Our log-likelihood analysis also suggested that another New Guinea fairy-wren, *M. cyanocephalus*, originated from Australian

ancestors (Fig. 2.3), though the distant relationships between this species and other *Malurus* taxa makes the timing of this event less clear.

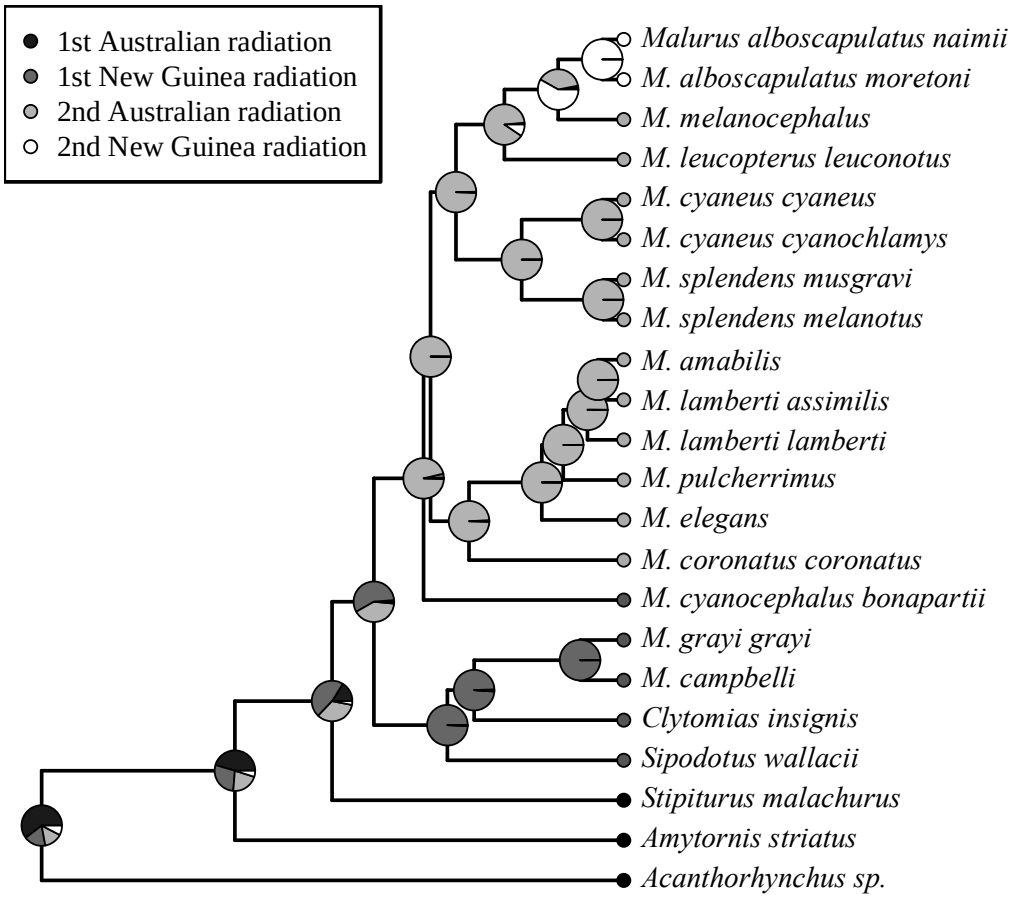

Figure 2.3. Ancestral reconstruction of geographic state of Maluridae using radiations described in Schodde (1982). Pie charts on notes represent the probability of each stated being the resolved ancestral state for that node. The category first Australian radiation is resolved as the ancestral state for the entire tree with a probability of 60.4%.

Relating dichromatism to latitude

A phylogenetically independent contrasts analysis comparing dichromatism (% of plumage character differences between the sexes) to mean breeding latitude revealed a

significant positive relationship (ANOVA: $F_{1,19} = 8.07$, $R^2 = 0.298$, $P = 0.010$; Fig. 2.4). This relationship was even stronger when we compared dichromatism to the lower latitudinal limit of each taxon's range ($F_{1,19} = 16.053$, $R^2 = 0.458$, $P = 0.001$). The farther south a taxon's geographical distribution extended, the more dichromatic the males and females in that taxon.

Three species provide interesting exceptions to the otherwise strong relationship between dichromatism and latitude in this group. The fairy-wren *M. cyanocephalus*, which appears to have dispersed from Australia to New Guinea (Fig. 2.3), is much more dichromatic than any other New Guinea fairy-wren (point A in Fig. 2.4). Conversely, the emu-wren (*Stiptiturus*) and grasswren (*Amytornis*) species included in our study are both notably less dichromatic than Australian *Malurus* taxa found at the same latitudes (points B and C, respectively, in Fig. 2.4).

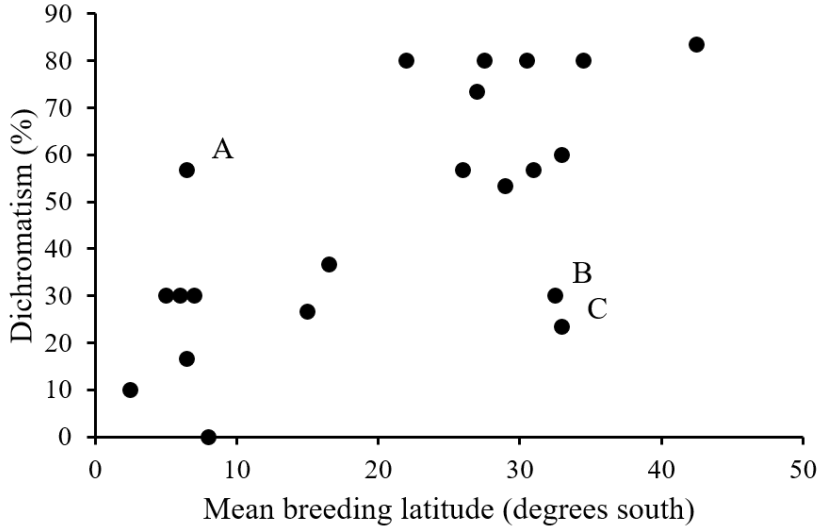

Figure 2.4. Pair-wise comparisons between dichromatism (percentage of plumage characters that differe between the sexes) and mean breeding latitude (degrees south, scored as the mid-point between northern and southern latitudinal limits of distribution) for 21 malurid taxa. Levels of dichromatism are significantly higher at more southerly latitudes in a phylogenetically independent contrasts analysis (ANOVA: $F_{1, 19} = 8.07$, $R^2 = 0.298$, $P = 0.010$). Points representing (A) *Malurus cyanocephalus*, (B) the emu-wrens *Stipiturus malachurus*, and (C) the grasswren *Amytornis striatus* are indicated.

DISCUSSION

For a relatively small family, the Maluridae show remarkable variation in plumage dichromatism. There are comparatively monochromatic species in which both males and females are relatively dull (emu-wrens) or relatively bright and conspicuous (New Guinea species of *Malurus*), and dichromatic species in which males are brighter than females (most *Malurus*) (Schodde 1982, Rowley and Russell 1997). Furthermore, at least one species (*M. alboscapulatus* in New Guinea) shows almost the entire range of possible levels of plumage dichromatism. In this species, males are invariant in their plumage (glossy black and white), whereas female plumage varies considerably across six populations. In one population (*M. alboscapulatus lorentzi*), females are dull brown: in two populations (*M. a. alboscapulatus* and *M. a. naimi*), females are pied in coloration (brown, black, and white); and in three populations (*M. a. aida*, *M. a.kutubu*, and *M. a. moretoni*), females are black and white, identical in plumage to males (Rowley and Russell 1997). This variation in plumage is similar to variation in other bird species in which plumage coloration is linked to aspects of biology such as mating system or breeding latitude (Prum 1990, 1997, Friedman et al. 2009, Price and Whalen 2009), making plumage variation in fairy-wrens potentially interesting.

Here we show that plumage characters in both males and females of species in Maluridae show similar overall numbers of changes during the evolutionary history of the group. Nevertheless, in some groups most plumage changes have occurred in males (e.g., the blue group) whereas in other groups most plumage changes have occurred in females (e.g., the chestnut-shouldered group). Importantly, these patterns of evolutionary change in plumage characters were similar regardless of whether we utilized the phylogeny of Driskell et al. (2011) or that of Lee et al. (2012).

Despite the fact that males and females show similar numbers of plumage changes and levels of homoplasy, the sexes nevertheless exhibited very different patterns of plumage evolution across the family. Differences between the plumage colors of males correlated well with genetic distance between species, whereas no such relationship existed for females (Fig. 2.2). This difference between the sexes was further emphasized by the results of the evolutionary models analysis, which indicated that plumage changes in males have accumulated steadily over time according to a Brownian Motion (BM) model. Plumage changes in females, in contrast, best fit an Ornstein Uhlenbeck (OU) model indicating natural selection, specifically a multi-optimum model with two adaptive peaks corresponding with New Guinea and Australia.

The steady divergence of male plumage colors in the Maluridae is strikingly similar to patterns of male secondary sexual trait evolution found in phylogenetic studies of some other avian clades. In the oropendolas (family Icteridae), for instance, both male plumage colors and male song characteristics have diverged along with genetic distance among taxa at surprisingly regular rates, with almost no examples of evolutionary convergence in these traits (Price and Lanyon 2002, Price and Whalen 2009). Likewise, in the manakins (family Pipridae), male plumage traits and display elements show similar patterns of divergence and low levels of homoplasy (Prum 1990, 1997). Both of these groups are highly polygynous and, like fairy-wrens, are presumably strongly influenced by sexual selection. Yet, why male traits in these taxa should show such remarkably constant and cumulative patterns of evolution is not clear (but see Prum 2010 for an intriguing explanation).

Unlike the steady divergence of male plumage patterns over time, female colors appear to have changed relatively little in some lineages and relatively dramatically in others, often resulting in large changes in dichromatism. For example, in both *Malurus amabilis* and *M.*

alboscapulatus, females independently gained bright coloration in their plumages and now look more like males than do the females of closely related taxa. In fact, in one subspecies of *M. alboscapulatus*, *M. a. moretoni*, males and females were identical in all plumage scores in our study. Both of these species have relatively northerly distributions in comparison to closely related taxa (Rowley and Russell 1997), so these rapid decreases in dichromatism appear to have occurred along with movements from higher to lower (more northern) latitudes.

Our results suggest that, during the history of the Maluridae, selection on plumage of females has been very different in New Guinea and Australia. This is corroborated by the positive correlation between percent dichromatism and breeding latitude. Dichromatism is generally greater in Australian species than it is in New Guinea species, and within Australia the species distributed farther south are more dichromatic than those species in northern regions. Moreover, all male fairy-wrens are brightly colored, whereas females tend to have male-like plumage in the north and duller, more cryptic colors in the south (Schodde 1982, Rowley and Russell 1997). The one exception to this pattern among the fairy-wrens, the New Guinea species *Malurus cyanocephalus*, has relatively high levels of dichromatism more similar to Australian congeners than to other New Guinea taxa (Fig. 2.4). This species also appears to have dispersed from Australia, based on our reconstruction of ancestral geographic ranges (Fig 4), which suggests that its high levels of dichromatism may be a retained ancestral state.

Two dramatic and related differences between the environments of New Guinea and Australia might relate to the latitudinal differences we observe in dichromatism. First, moving south into Australia from New Guinea, there is the gradient from tropical forests, to temperate forests, to savannah, and finally to open grasslands (Rowley and Russell 1997). Second, there is the gradient towards greater environmental seasonality moving south. This occurs both in

comparing New Guinea to Australia and also within the continent of Australia. Whether it is habitat structure, seasonality, or a combination of the two that has yielded selection pressures on females for different levels of crypsis is unknown. Nevertheless, we suggest that it is directly or indirectly related to predation at nests and the advantages of crypsis for incubating females (Martin and Badyaev 1996).

Interestingly, emu-wrens and grasswrens differ from this general latitudinal pattern in that both males and females in these species are dull colored and less dichromatic than fairy-wrens at similar latitudes (Fig. 2.4). Evidence suggests that these taxa also have much lower rates of extra-pair copulation than do Australian fairy-wrens (Rowe and Pruett-Jones 2012), which may explain the lack of bright colors in males (Webster et al. 2007). However, the potential influences of sexual selection and ecological factors in explaining these relatively low levels of dichromatism remain to be investigated, perhaps in comparative studies including additional emu-wren and grasswren taxa.

In a study similar to ours, but focusing primarily on female fairy-wrens, Karubian (2013) scored plumage ornamentation as high, moderate, or low and also showed that female ornamentation increased in species whose distributions were closer to the equator (lower latitudes). In keeping with our results that plumage changes in female malurids fit an evolutionary model indicating selection, Karubian (2013) also argues that plumage ornamentation in females is likely the result of selection rather genetic correlations with males or other factors. Karubian (2013) also examines variation in bill coloration across fairy-wrens and shows that it is substantially more variable in females than in males, and that, as with plumage, some of the variation is associated with geography (tropical species have darker bills than species in temperate areas).

Our result that dichromatism varied with latitude adds to an existing literature showing that patterns of dichromatism vary between tropical and temperate regions (Hamilton 1961, Price and Birch 1996, Friedman et al. 2009). In these previous studies, however, it wasn't latitude *per se* that was the focus of the association, but rather migratory behavior of the species. For example, using phylogenetic comparative methods, Friedman et al. (2009) reported a statistically significant association between the evolution of dichromatism and migratory behavior in New World orioles (*Icterus*), due to losses of bright plumage in the females of migratory, temperate breeding taxa. Our data show similar patterns in *Malurus*, in which different levels of dichromatism occur at different latitudes, largely due to changes in female plumage. Nevertheless, fairy-wrens are not migratory, and thus selection associated with migration cannot explain our findings. In both fairy-wrens and orioles, relatively dichromatic species occupy habitats with greater seasonality and relatively monochromatic species are found in the tropics (Rowley and Russell 1997, Jaramillo and Burke 1999). Friedman et al. (2009) argue that elaborate plumage in females may be advantageous in sedentary species and possibly maladaptive in migratory species. However, it could be that differences in female oriole plumage are better explained by differences between tropical and temperate habitats. These alternatives cannot be distinguished in the oriole group, in which all temperate breeding species are also migratory (Jaramillo and Burke 1999). Thus, our data for fairy-wrens suggest that migration *per se* may not be as important in plumage evolution as Friedman et al. (2009) suggest.

In this study, we visually scored the plumages of males and females of each species using methods similar to those used in other recent studies of color evolution (Irwin 1994, Burns 1998, Omland and Lanyon 2000, Price and Whalen 2009). Nevertheless, it is now well known that ultraviolet light is important in the plumage and vision of birds (Cuthill et al. 2000). In a recent

study, Eaton (2005) reports that >90% of 139 presumably monochromatic species are, in fact, dichromatic based on avian vision and plumage reflectance data. These results suggest that most 'visually' monochromatic bird species are likely dichromatic as perceived by birds (Eaton 2005) and argue that many of our standard paradigms concerning avian plumage evolution may be incorrect. We did not assess ultraviolet reflectance in our study, and we acknowledge that some of the species we categorized as relatively monochromatic may be more dichromatic than our subjective scores indicate. Nevertheless, our measures presumably reflect the wide range in relative levels of dichromatism characteristic of the Maluridae.

Plumage colors in the Maluridae, as in any bird family, are a result of complex interactions between evolutionary history, ecology, and sexual selection (Bennett and Owens 2002, Badyaev and Hill 2003). It is clear, however, that regardless of selection pressures affecting males (e.g., sexual selection), changes in female plumage are equally important in generating current patterns of dichromatism. Our evolutionary model analyses suggest that males and females in the Maluridae are often under different selective pressures and that these have had significant impacts on patterns of species divergence. Our results strengthen previous suggestions (Irwin 1994, Wiens 2001, Hofmann et al. 2008, Friedman et al. 2009) that when researchers are studying dichromatic species in which males are bright and females dull, they should not immediately assume that this is the result of sexual selection in males. In fairy-wrens, we know that sexual selection represents a significant selective pressure on traits in males (Webster et al. 2007, Rowe and Pruett-Jones 2006, 2011, 2012), but sexual selection does not, in fact, appear to be solely responsible for the observed patterns of dichromatism in *Malurus*.

More broadly, our results highlight the importance of incorporating models of evolution with phylogenetic analyses of present and past character states. Our phylogenetic analyses, by

themselves, showed that plumage in both males and females in malurid species were changing at approximately the same rates on average, but it was the evolutionary models analysis that indicated it was females that were more often under stronger directional selection, either for increased or decreased plumage elaboration. With the increasing ease of incorporating such evolutionary models analyses in research, we believe that future studies will confirm the importance of selection on plumage in females as a major force underlying existing patterns of dichromatism in birds.

APPENDIX 2A: PLUMAGE SCORES FOR MALURIDAE SPECIES

Table 2.A.1. Plumage scores for male fairy-wren, grasswren, and emu-wren (Maluridae) species, as well as *Acanthorhynchus tenuirostris*. Color character state definitions are: B, blue; C, carotenoid; N, black; W, white; D, dark brown; L, light brown. Other character states are: 0, absent; 1, present.

	Crown	Black Crown Spot	Supercillium	Superloral	Eye-line	Eye-ring	Lores
Malurus splendens melanotus	B	0	B	B	1	B	N
M. splendens musgravi	B	0	B	B	1	B	N
M. coronatus coronatus	B	1	B	B	1	N	N
M. cyaneus cyaneus	B	0	B	B	1	B	N
M. cyaneous cyanochlamys	B	0	B	B	0	B	N
M. lamberti lamberti	B	0	B	B	0	B	N
M. lamberti assimilis	B	0	B	B	0	B	N
M. pulcherrimus	B	0	B	B	0	B	N
M. amabilis	B	0	B	B	0	B	N
M. elegans	B	0	B	B	0	B	N
M. leucopterus leuconotus	B	0	B	B	0	B	B
M. melanocephalus	N	0	N	N	0	N	N
M. alboscapulatus naimii	N	0	N	N	0	N	N
M. alboscapulatus moretoni	N	0	N	N	0	N	N
M. cyanocephalus bonapartii	B	0	N	N	1	N	N
Sipodotus wallacii	N	0	N	N	1	W	N
Clytomias insignis	C	0	C	C	0	C	C
M. grayi grayi	N	0	B	B	1	B	N
M. campbelli	N	0	B	B	1	B	N
Stipiturus malachurus	C	0	B	C	0	W	C
Amytornis striatus	C	0	C	C	0	W	C
Acanthorhynchus tenuirostris	N	0	N	N	1	N	N

Table 2.A.1. CONTINUED

	Aricular	Throat	Malar	Breast	Upper Belly	Lower Belly	Nape
Malurus splendens melanotus	B	B	N	N	B	w	N
M. splendens musgravi	B	B	N	N	B	B	N
M. coronatus coronatus	N	W	W	W	W	W	N
M. cyaneus cyaneus	B	N	N	N	W	W	N
M. cyaneous cyanochlamys	B	N	N	N	W	W	N
M. lamberti lamberti	B	N	N	N	W	W	N
M. lamberti assimilis	B	N	N	N	W	W	N
M. pulcherrimus	B	B	N	N	W	W	N
M. amabilis	B	N	N	N	W	W	N
M. elegans	B	B	N	N	W	W	N
M. leucopterus leuconotus	B	B	B	B	B	B	B
M. melanocephalus	N	N	N	N	N	N	N
M. alboscapulatus naimii	N	N	N	N	N	N	N
M. alboscapulatus moretoni	N	N	N	N	N	N	N
M. cyanocephalus bonapartii	N	B	B	B	B	B	N
Sipodotus wallacii	W	W	N	W	W	W	C
Clytomias insignis	C	C	C	C	C	C	C
M. grayi grayi	B	B	N	B	B	B	N
M. campbelli	B	B	N	B	B	B	N
Stipiturus malachurus	C	B	C	B	L	W	D
Amytornis striatus	D	W	N	L	L	L	C
Acanthorhynchus tenuirostris	N	C	W	W	N	L	C

Table 2.A.1. CONTINUED

	Scapulars	Mantel	Back	Tail	Tail Edge	Wing Coverts	Wing Covert Edges
Malurus splendens melanotus	N	B	N	B	W	B	B
M. splendens musgravi	N	B	N	B	W	B	B
M. coronatus coronatus	D	D	D	B	B	D	D
M. cyaneus cyaneus	N	B	N	B	B	N	N
M. cyaneous cyanochlamys	N	B	N	N	N	N	N
M. lamberti lamberti	C	B	N	B	B	D	D
M. lamberti assimilis	C	B	N	B	B	D	D
M. pulcherrimus	C	B	N	B	B	D	D
M. amabilis	C	B	N	B	W	D	D
M. elegans	C	B	N	B	B	D	D
M. leucopterus leuconotus	W	B	B	B	B	W	W
M. melanocephalus	C	C	C	N	N	N	N
M. alboscapulatus naimii	W	N	N	N	N	N	N
M. alboscapulatus moretoni	W	N	N	N	N	N	N
M. cyanocephalus bonapartii	B	B	N	B	B	B	B
Sipodotus wallacii	C	C	C	D	W	D	W
Clytomias insignis	D	D	D	C	C	C	C
M. grayi grayi	B	B	B	D	D	D	D
M. campbelli	D	D	B	D	D	D	D
Stipiturus malachurus	D	D	D	D	D	D	D
Amytornis striatus	C	C	C	D	D	D	D
Acanthorhynchus tenuirostris	D	D	D	D	W	D	D

Table 2.A.1. CONTINUED

	Secondary	Secondary Tip	Primary	Beak	Flanks	Iridescence	Crown Streaked Blue
Malurus splendens melanotus	B	B	B	N	B	1	0
M. splendens musgravi	B	B	B	N	B	1	0
M. coronatus coronatus	D	D	D	N	L	1	0
M. cyaneus cyaneus	N	N	N	N	W	1	0
M. cyaneous cyanochlamys	N	N	N	N	W	1	0
M. lamberti lamberti	D	D	D	N	L	1	0
M. lamberti assimilis	D	D	D	N	W	1	0
M. pulcherrimus	D	D	D	N	L	1	0
M. amabilis	D	D	D	N	W	1	0
M. elegans	C	C	D	N	L	1	0
M. leucopterus leuconotus	W	W	D	N	B	1	0
M. melanocephalus	N	N	N	N	N	0	0
M. alboscapulatus naimii	N	N	N	N	N	0	0
M. alboscapulatus moretoni	N	N	N	N	N	0	0
M. cyanocephalus bonapartii	B	B	N	N	B	1	0
Sipodotus wallacii	D	D	D	N	W	0	1
Clytomias insignis	C	C	C	N	C	0	0
M. grayi grayi	D	D	D	N	B	1	0
M. campbelli	D	D	D	N	B	1	0
Stipiturus malachurus	D	D	D	N	L	0	0
Amytornis striatus	D	D	D	N	L	0	0
Acanthorhynchus tenuirostris	D	D	D	N	L	0	0

Table 2.A.1. CONTINUED

	White Streaking	Black Streaking
Malurus splendens melanotus	0	0
M. splendens musgravi	0	0
M. coronatus coronatus	0	0
M. cyaneus cyaneus	0	0
M. cyaneous cyanochlamys	0	0
M. lamberti lamberti	0	0
M. lamberti assimilis	0	0
M. pulcherrimus	0	0
M. amabilis	0	0
M. elegans	0	0
M. leucopterus leuconotus	0	0
M. melanocephalus	0	0
M. alboscapulatus naimii	0	0
M. alboscapulatus moretoni	0	0
M. cyanocephalus bonapartii	0	0
Sipodotus wallacii	0	0
Clytomias insignis	0	0
M. grayi grayi	0	0
M. campbelli	0	0
Stipiturus malachurus	1	1
Amytornis striatus	1	0
Acanthorhynchus tenuirostris	0	0

Table 2.A.2. Plumage scores for female fairy-wren, grasswren, and emu-wren species (Maluridae), as well as *Acanthorhynchus tenuirostris*. The ordering of taxa follow Rowley and Russell (1997). Color character state definitions are: B, blue; C, carotenoid; N, black; W, white; D, dark brown; L, light brown. Other character states are: 0, absent; 1, present.

	Crown	Black Crown Spot	Supercillium	Superloral	Eye-line	Eye-ring	Lores
Malurus splendens melanotus	D	0	D	D	0	C	C
M. splendens musgravi	D	0	D	D	0	C	C
M. coronatus coronatus	D	0	L	D	0	W	D
M. cyaneus cyaneus	D	0	D	D	0	C	C
M. cyaneous cyanochlamys	D	0	D	D	0	C	C
M. lamberti lamberti	D	0	D	D	0	C	C
M. lamberti assimilis	D	0	D	D	0	C	C
M. pulcherrimus	D	0	D	D	0	C	C
M. amabilis	B	0	B	B	0	W	W
M. elegans	D	0	D	D	0	D	C
M. leucopterus leuconotus	D	0	D	D	0	D	D
M. melanocephalus	D	0	D	D	0	L	L
M. alboscapulatus naimii	N	0	N	W	0	W	N
M. alboscapulatus moretoni	N	0	N	N	0	N	N
M. cyanocephalus bonapartii	B	0	N	N	0	N	N
Sipodotus wallacii	N	0	N	N	0	W	W
Clytomias insignis	C	0	C	C	0	C	C
M. grayi grayi	N	0	B	B	0	B	N
M. campbelli	N	0	B	B	0	B	N
Stipiturus malachurus	D	0	D	D	0	W	L
Amytornis striatus	D	0	D	C	0	W	C
Acanthorhynchus tenuirostris	D	0	D	D	0	N	D

Table 2.A.2. CONTINUED

	Aricular	Throat	Malar	Breast	Upper Belly	Lower Belly	Nape
Malurus splendens melanotus	L	W	W	W	W	L	D
M. splendens musgravi	L	W	W	W	W	L	D
M. coronatus coronatus	D	W	W	W	W	W	D
M. cyaneus cyaneus	L	W	W	W	W	L	D
M. cyaneous cyanochlamys	L	W	W	W	W	L	D
M. lamberti lamberti	D	W	W	W	W	L	D
M. lamberti assimilis	D	W	W	W	W	L	D
M. pulcherrimus	D	L	L	L	L	L	D
M. amabilis	B	W	W	W	W	W	B
M. elegans	D	W	W	W	W	L	D
M. leucopterus leuconotus	D	W	W	W	W	L	D
M. melanocephalus	D	W	W	W	W	L	D
M. alboscapulatus naimii	N	W	W	P	P	W	N
M. alboscapulatus moretoni	N	N	N	N	N	N	N
M. cyanocephalus bonapartii	N	B	N	W	W	L	B
Sipodotus wallacii	W	L	N	L	L	L	N
Clytomias insignis	C	L	C	C	C	L	C
M. grayi grayi	B	B	N	B	W	W	N
M. campbelli	B	B	N	B	W	W	N
Stipiturus malachurus	W	L	L	L	L	W	D
Amytornis striatus	D	W	N	L	L	L	D
Acanthorhynchus tenuirostris	D	D	D	L	L	L	C

Table 2.A.2. CONTINUED

	Scapulars	Mantel	Lower Back	Tail	Tail edge	Wing coverts	Wing covert edges
Malurus splendens melanotus	D	D	D	B	B	D	D
M. splendens musgravi	D	D	D	B	B	D	D
M. coronatus coronatus	D	D	D	B	B	D	D
M. cyaneus cyaneus	D	D	D	D	D	D	D
M. cyaneous cyanochlamys	D	D	D	D	D	D	D
M. lamberti lamberti	D	D	D	B	B	D	D
M. lamberti assimilis	D	D	D	B	B	D	D
M. pulcherrimus	D	D	D	B	B	D	D
M. amabilis	B	B	B	B	W	D	D
M. elegans	C	D	D	D	D	D	D
M. leucopterus leuconotus	D	D	D	B	B	D	D
M. melanocephalus	D	D	D	D	D	D	D
M. alboscapulatus naimii	W	N	N	N	N	N	W
M. alboscapulatus moretoni	W	N	N	N	N	N	N
M. cyanocephalus bonapartii	C	C	C	N	W	D	D
Sipodotus wallacii	C	C	C	D	W	D	W
Clytomias insignis	D	D	D	D	D	D	D
M. grayi grayi	B	B	B	D	D	D	D
M. campbelli	B	B	B	D	D	D	D
Stipiturus malachurus	D	D	D	D	D	D	D
Amytornis striatus	D	D	D	D	D	D	D
Acanthorhynchus tenuirostris	D	D	D	D	D	D	D

Table 2.A.2. CONTINUED

	Secondary	Secondary Tip	Primary	Beak	Flanks	Iridescence	Crown Streaked Blue
Malurus splendens melanotus	D	D	D	C	L	0	0
M. splendens musgravi	D	D	D	C	L	0	0
M. coronatus coronatus	D	D	D	N	L	0	0
M. cyaneus cyaneus	D	D	D	C	L	0	0
M. cyaneous cyanochlamys	D	D	D	C	L	0	0
M. lamberti lamberti	D	D	D	C	L	0	0
M. lamberti assimilis	D	D	D	C	L	0	0
M. pulcherrimus	D	D	D	C	L	0	0
M. amabilis	D	D	D	N	W	1	0
M. elegans	D	D	D	N	L	0	0
M. leucopterus leuconotus	D	D	D	C	L	0	0
M. melanocephalus	D	D	D	C	L	0	0
M. alboscapulatus naimii	N	W	N	N	N	0	0
M. alboscapulatus moretoni	N	N	N	N	N	0	0
M. cyanocephalus bonapartii	D	D	D	N	C	1	0
Sipodotus wallacii	D	D	D	N	L	0	1
Clytomias insignis	D	D	D	N	C	0	0
M. grayi grayi	D	D	D	N	B	1	0
M. campbelli	D	D	D	N	B	1	0
Stipiturus malachurus	D	D	D	N	L	0	0
Amytornis striatus	D	D	D	N	C	0	0
Acanthorhynchus tenuirostris	D	D	D	N	L	0	0

Table 2.A.2. CONTINUED

	White Streaking	Black Streaking
Malurus splendens melanotus	0	0
M. splendens musgravi	0	0
M. coronatus coronatus	0	0
M. cyaneus cyaneus	0	0
M. cyaneous cyanochlamys	0	0
M. lamberti lamberti	0	0
M. lamberti assimilis	0	0
M. pulcherrimus	0	0
M. amabilis	0	0
M. elegans	0	0
M. leucopterus leuconotus	0	0
M. melanocephalus	0	0
M. alboscapulatus naimii	0	0
M. alboscapulatus moretoni	0	0
M. cyanocephalus bonapartii	0	0
Sipodotus wallacii	0	0
Clytomias insignis	0	0
M. grayi grayi	0	0
M. campbelli	0	0
Stipiturus malachurus	0	1
Amytornis striatus	1	0
Acanthorhynchus tenuirostris	0	0

CHAPTER 3: HETEROSPECIFIC SOCIALITY MEDIATED BY SONG DISCRIMINATION IN FAIRY-WRENS

ABSTRACT

In many territorial species, individuals discriminate between conspecifics on the basis of their proximity and potential threat. Neighbor-stranger discrimination is common in territorial animals including birds, mammals, amphibians and insects (Ydenberg et al. 1988, Temeles 1994). Individuals of different species may also interact competitively (Goodale et al. 2010). In such cases, discrimination between heterospecific rivals and non-rivals may be beneficial, allowing individuals to appropriately direct aggression. Evidence for neighbor-stranger discrimination across species has previously been demonstrated in ants (Langen et al. 2000). Here we provide evidence of heterospecific discrimination of rivals in vertebrate species. In variegated and splendid fairy-wrens (*Malurus lamberti* and *M. splendens*) family groups often live on overlapping territories where the species' distributions overlap. As a result, each family group has heterospecific "co-residents" with whom they defend a shared territory. We found that individuals of both species responded more aggressively to the songs of neighboring and foreign heterospecific fairy-wrens than they did to the songs of their co-resident heterospecifics. Variegated fairy-wrens exhibited an increased duration of foraging, decreased vigilance, and were more likely to attempt to re-nest when associating closely with splendid fairy-wrens. These findings suggest cooperation may occur between these two species on joint territories, mediated by vocal discrimination of heterospecific individuals. Heterospecific group member recognition and selective cooperation represents another mechanism through which species interactions drive ecology and behavior.

INTRODUCTION

Recognition is an important component for both cooperative and competitive social behavior. For example, mate or group member recognition facilitates cooperative social behaviors by ensuring that individuals do not direct potentially costly behaviors to inappropriate individuals (Temeles 1994). Discrimination between neighbors and strangers allows individuals to adjust their aggressive responses based on the potential threat posed by familiar and unfamiliar rivals. A special case of neighbor-stranger discrimination, the "dear enemy" effect (Fisher 1954), in which individuals display lowered aggression to neighboring individuals than to unfamiliar individuals, helps reduce time and energy wasted defending a territory from an established territory holder (Temeles 1994). Examples of recognition and discrimination of individuals mediating social interactions have been documented in many systems (Stoddard et al. 1990, Clark et al. 2006, Tibbetts and Dale 2007, Masco 2013).

Heterospecific interactions, like conspecific sociality, can influence species' ecology and distribution through competition, cooperation, predation, or attraction (in which species made site choice decisions based on the presence of another species; Mönkkönen and Forsman 2002, Seppänen et al. 2007, Valone 2007, Goodale et al. 2010, Wheatcroft and Price 2013). While most heterospecific associations or social interactions are transient and not specific to individuals, long-term interactions between specific individuals have been observed in several systems (Powell 1985, Grutter and Bshary 2003, Harrison and Whitehouse 2011, Vail et al. 2014). How long-term heterospecific relationships are mediated is poorly understood (Goodale et al. 2010). One possibility is recognition of and discrimination among heterospecific individuals. When heterospecific interactions are long-term or specific to particular individuals, as in co-defended territories found in some tropical bird species (Munn and Terborgh 1979), such

recognition and discrimination may be beneficial. The dear-enemy effect has been described in ants, and is hypothesized to help direct antagonistic interactions between neighbor and stranger workers that pose differing threat levels to colony resources (Heinze et al. 1996, Langen et al. 2000, Tanner and Adler 2009). While the presence of such recognition has been investigated in a least one vertebrate species pair, the rufous-and-white wren (*Thryophilus rufalbus*) and the banded wren (*T. pleurostictus*) no discrimination between heterospecific neighbors or strangers was found, and as far as we are aware such discrimination has yet to be found in any vertebrate species despite the frequency of heterospecific interactions (Battiston et al. 2015).

The variegated and splendid fairy-wrens (*Malurus lamberti* and *M. splendens*, respectively) are small (7-10 g), cooperatively breeding passerines endemic to Australia (Rowley and Russell 1997). These species co-occur over part of their distribution and, when sympatric, they occur on partially or fully overlapping territories (Tibbets and Pruett-Jones 1999). In such cases, individuals of both species often travel and forage together, appearing to behave aggressively toward the co-residents (the heterospecific social group with whom they share a territory) only when close to a nest (Johnson personal observation). Despite this tolerant behavior, both species exhibit territorial behavior towards *non*-co-resident conspecific and heterospecific fairy-wrens. If interactions are beneficial and coalitions are being formed with specific individuals, we hypothesized that both species would discriminate between co-resident and other individuals of the heterospecific. Here we use song playbacks to show this is the case. Because discrimination between heterospecific individuals could be the result of habituation rather than because of cooperation and recognition, we also monitored time budgeting and breeding of both species to determine if heterospecific associations alter species behavior or fecundity. We also performed conspecific neighbor-stranger experiments as recognition studies

have not been performed in either species or in related species (Maluridae) to determine if neighbor-stranger discrimination is expected. This is the first known example of discrimination among different heterospecific individuals in a wild vertebrate system.

METHODS

Study system

We conducted behavioral and experimental studies on variegated and splendid fairy-wrens at Brookfield Conservation Park, South Australia (S 34°21', E 139°29'). The park is characterized by mallee eucalyptus and chenopod scrub. Although both species have been studied at this site since 1992 (Tibbets and Pruett-Jones 1999, Grieg et al. 2010, Pruett-Jones et al. 2010), our intensive monitoring of overlapping territories, and interacting social groups took place from 2011-2015. During these years we color banded all adult members of each social group for both splendid and variegated fairy-wrens. Each adult was banded with a unique combination of three color bands (combinations were repeated in males and females as they are distinguishable by plumage) and a numbered metal band issued by the Australian Bird and Bat Banding Scheme. Unique band combinations allowed individuals to be recognized and relationships between individuals to be determined.

Both splendid and variegated fairy-wrens are cooperative breeders, a breeding system characterized by delayed dispersal of young who assist the adults in rearing offspring in subsequent nests. At Brookfield Conservation Park, the variegated fairy-wren is highly social, living in groups from 2-9 birds (see Chapter 4). While the splendid fairy-wren is also cooperative, the presence of helpers at this site is rare.

Ethical note

Capture and banding of variegated and splendid fairy-wrens for individual identification as well as behavioral observations and behavioral manipulation of both species were supported by approvals from the University of Chicago Animal Care and Use Committee (ACUP permit number 72322 and 72273) and the South Australian Animal Ethics Committee (Wildlife Ethics Committee approvals 33-2015 and 21-2013).

Territory overlap and breeding success

To determine if sharing a territory with a heterospecific group influences breeding success we monitored reproduction of both species during the breeding season (September to December). Each group that initiated breeding (completed a nest) was monitored for nest success. Between 2013 and 2015 we monitored nesting and breeding behavior of variegated and splendid fairy-wrens on solitary (no co-resident) and shared territories (a territory overlapping with the other fairy-wren species). Territories were considered shared if each species' territory contained the nest of the other species, if they shared more than 50% of the territory area, and if they were observed to forage or travel with the co-resident. In all cases of unquestionable territory overlap all three conditions were met. Only groups that attempted to breed at least once were included in this study for a total of 49 splendid and 33 variegated fairy-wren social groups in 2013, 58 splendid and 60 variegated fairy-wren social group groups in 2014, and 38 splendid and 54 variegated fairy-wren social groups in 2015. Of the splendid fairy-wren groups, 60% shared territories with variegated fairy-wrens, and a comparable 65% of variegated fairy-wren territories were shared with splendid fairy-wrens. For each group that attempted to breed we

recorded if they 1) fledged young from their first initiated nest, 2) fledged young at any point during the field season, and 3) if they initiated re-nesting behavior following initial nest failure. Group size correlates positively with fledging success in variegated fairy-wrens (Johnson and Pruett-Jones, in prep). However, average group size of variegated fairy-wrens did not significantly differ between shared and solitary territories (N= 147, ANOVA, F = 1.459, P = 0.23), and was thus ignored in all further analyses. We used chi-squared tests to determine if frequencies fledging and re-nesting differed on solitary or shared territories for each species. Groups were excluded from the first analysis (fledged young from first initiated nest) if the group fledged young but we did not know if it was from the first nest (N retained = 154, 125 for splendid and variegated fairy-wrens respectively). Groups were excluded from the second analysis (fledged young) if at the end of the season we were unable to determine if the final nest had fledged young (N retained = 147, 145 for splendid and variegated fairy-wrens respectively). Groups were excluded from the re-nesting analysis if they nested once but the group was not followed closely enough to determine if re-nesting was attempted (N retained = 127, 139 for splendid and variegated fairy-wrens respectively).

Time budgeting in shared territories

In 2014 we performed 'focal follows' of 11 splendid and 12 variegated fairy-wren social groups on shared territories to examine whether the behavior of each species changed in the presence of the other. All splendid fairy-wren groups were monitored for this aspect of the study consisted of a dominant pair with no auxiliary group members (group size = 2.0), whereas most variegated fairy-wren did have auxiliary group members (average group size of 3.08 ± 0.31 S. E.). We attempted to complete 120 min of focal follows, however, due to the difficulty of

tracking, each group was followed for an average of 113.5 min (± 15.776 S.D.). We recorded the behavior of the dominant male of the focal group at 1-min intervals. Behaviors were partitioned into foraging, traveling, singing, contact calling, and mobbing. We also recorded vigilance behavior (posting high up, in the open looking around) of any individual in the social group as a binary variable (yes or no) during each minute of the focal follow, regardless of behavior recorded at the 1-min scan sample. While not all shared territories were included in both species focal follows, for those that were we monitored each species on separate occasions. We used paired t-tests to compare each behavior during periods alone and with the co-resident, and combined the *P*-values across behaviors using the Z-transform method (Whitlock 2005).

Playback experiments

Heterospecific group member and neighbor stranger discrimination

We conducted a set of reciprocal playback experiments on coincident territories to test whether individuals discriminate between co-resident and non-co-resident heterospecifics and if they discriminate between neighboring and foreign heterospecifics on the basis of song. In 2013, we conducted playback trials on a total of 15 variegated fairy-wren social groups that had territories coincident with a splendid fairy-wren group. We presented male variegated fairy-wrens with songs of the male splendid fairy-wrens that: 1) occupied the same territory (co-resident treatment), 2) occupied an adjacent territory (neighbor treatment), and 3) occupied an area 5 or more territories away (foreign treatment). While most splendid fairy-wren social groups did not contain auxiliary members, one shared territory contained a subordinate male, and at least one stranger stimulus was recorded from males in territories with a subordinate male. In these cases, the male song presented as the stimuli was the dominant male (breeding male). Each male

also received 4) a control treatment consisting of the songs of a heterospecific species that both fairy-wren species frequently encounter but from whom they do not defend their territories: the red-capped robin (*Petroica goodenovii*). Treatment 2 thus served as a known intruder, while treatment 3 is a presumed unknown intruder. In 2014, we performed the reciprocal experiment, conducting playback trials on a total of 15 splendid fairy-wren groups that shared territories with variegated fairy-wrens. Male splendid fairy-wrens were presented with the same four treatment types, using the songs of dominant variegated males as the stimuli in treatments 1-3.

To create playback stimuli, we recorded dawn chorus songs of males of both species during the same year the experiment was completed. All recordings were made using Sennheiser ME66 and ME67 shotgun microphones and Marantz PMD-661 digital recorders. Songs were recorded as uncompressed wav files at a sample rate of 44100 Hz. Using Syrinx-PC (J. Burt, Seattle, WA, U.S.A.) we edited these recordings to produce playback stimuli, each consisting of eight songs with five sec of silence between each song (for a total duration of ~1 min). The maximum amplitude of each song was standardized. The amplitude of each stimulus was measured in the field using a Pyle PSPL01 Mini Digital Sound Level Meter (SPL, Pyle Audio Inc., Brooklyn, NY) to ensure that the playback volume was kept constant across trials. The field amplitude for all playback stimuli was approximately 87.0 dB SPL at a distance of 1 m from the speaker.

The playback experiments were conducted in the morning (0700-1100) while the birds were visible on their territories. The stimuli were played in the field using a Saul Mineroff Electronics amplified field speaker (Saul Mineroff Electronics Inc., Elmont, NY) and an iPod Classic (Apple Inc., Cupertino, CA). The speaker was positioned on the ground approximately

30 m from the focal bird. For each trial, the stimulus was played three times with five minutes of observation in between, yielding a total trial duration of 18 minutes.

Each focal group was presented with each of the four treatments, one per morning for four mornings, in a random order. While we attempted to have all treatments presented on consecutive mornings, high wind prevented this in a few cases. When this occurred the focal group was presented with the next treatment on a subsequent morning. During the 18-min trial the response variables latency to first response was recorded, as well as the number of occurrences of the response variables "approach", "songs," "duration of vigilance," and "scold." The response variable "songs" includes both Type I and Type I+II songs sung by both males and females, which are song types hypothesized to function in territorial defense (Greig and Pruett-Jones 2008). The songs and other response variables are typical aggressive responses to territorial intruders (Greig and Pruett-Jones 2008). As these response variables are all measures of aggression and were strongly intercorrelated (with $r > |0.3|$ for at least one pairwise correlation for each variable of variegated fairy-wren response and $r > |0.3|$ for at least one pairwise correlation for each variable of splendid fairy-wren response) we performed a principle component analysis (PCA) to derive a single composite score that was used in subsequent analyses (McGregor and Avery 1986). PCA was performed separately for each species to account for species-specific response behavior (Table 3.A.1). Splendid fairy-wren responses all loaded heavily on PC1, and two responses loaded onto PC2, however, because all responses were present on PC1, this was used in all subsequent analyses. Variegated fairy-wren response variables all loaded strongly onto PC1. An additional variable was recorded for splendid fairy-wrens, the Type II song. The Type II song is a predator-elicited vocalization sung by males that is thought to function as a sexual display to conspecific females (Greig and Pruett-Jones 2010,

Greig et al. 2010). We therefore expected male fairy-wrens to produce Type I or Type I+II songs but not just Type II songs in response to intrusion. While both splendid and variegated fairy-wrens produce Type II songs, variegated fairy-wrens sing Type II songs very infrequently and were not observed to produce them during the experiment. As such, the number of Type II songs produced was only examined for splendid fairy-wrens.

Conspecific neighbor-stranger discrimination

Because neighbor-stranger discrimination has not been studied in any *Malurid* species, we performed two playback experiments, one on each species, to determine if conspecific neighbor-stranger discrimination occurs. Males of each species were presented with songs of dominant male conspecific from 1) a neighboring territory and 2) a foreign territory (five or more territories away) as well as 3) a control song, the red-capped robin, which they regularly hear but to which they do not respond. Stimuli production and presentation time were the same as described in the heterospecific playback experiments. However, rather than being played within the territory as the heterospecific stimuli were, conspecific stimuli were played from the territory border of the neighbor stimulus and were used to indicate an intrusion threat, as none of the stimuli would normally be experienced by the focal group from within their territory.

Responses to stimuli differed slightly from those recorded for the heterospecific playback experiment. Some behaviors were exclusive to conspecific interactions and one (look) was easier to observe during trials as there was a stronger response for this category for conspecific interactions. The response variables for the conspecific playback included “latency to first response,” “number of approaches to the speaker” (approach), “bill-wipe,” “mate-guarding” (which included display behaviors directed at the female, as well as chasing, or finding the

female), “look,” “sing,” “duration of vigilance,” and “scold.” All response variables were correlated with at least one other variables ($r > 0.4$), save for scold, which did not vary across treatments and was thus removed from the analysis. Again, we performed a PCA on each species separately (Table 3.A.2). Splendid fairy-wren response variables all loaded strongly onto PC1. Variegated responses all loaded heavily on PC1, and three responses loaded onto PC2, however, because all responses were present on PC1 this was used in all subsequent analyses.

RESULTS

Breeding success

For both species there was no difference in fledging success over the breeding season on solitary or shared territories ($\chi^2 = 2.64, 0.01$; $P = 0.10, 0.92$ for splendid and variegated fairy-wrens, respectively; see Table 3.B.1 for sample sizes), nor was there any difference in the fledging success of the first nest or re-nesting behavior for splendid-fairy-wrens ($\chi^2 = 1.60, 0.72$; $P = 0.14, 0.10$ for splendid and variegated fairy-wrens, respectively; Table 3.B.1). However, more variegated fairy-wren groups occupying shared territories succeeded in fledging their first nest ($\chi^2 = 5.97$, $P = 0.02$, Figure 3.1) and attempted to re-nest following initial nest failure, than did those on solitary territories ($\chi^2 = 6.99$, $P < 0.01$).

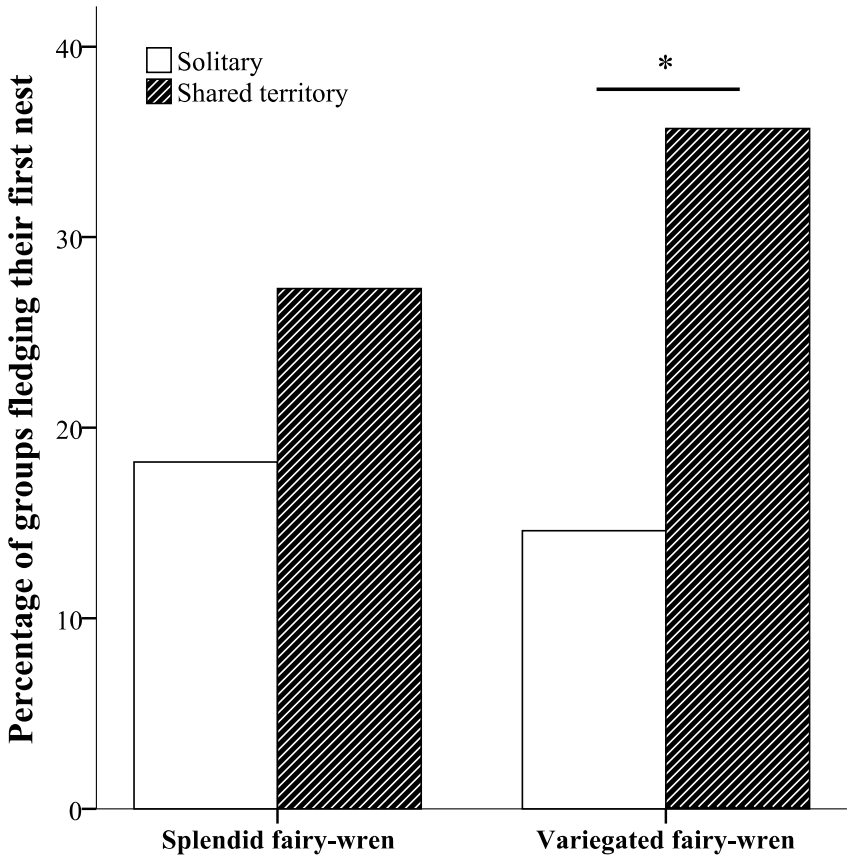

Figure 3.1. Percentage of groups successfully fledging young from their first nesting attempt by territory status (solitary or shared with a heterospecific social group), grouped by species. Solitary territories are indicated by clear bars, shared territories by hashed bars. * indicates $P < 0.05$. Analyses are separate for each species.

Time budgeting in shared territories

Variegated and splendid fairy-wrens spent on average 45% of their time within 20 m of their co-resident heterospecific group. Splendid fairy-wrens did not change their behavior when interacting with variegated fairy-wrens (Z-transform test Z = -0.61, $P = 0$ .27, for independent test p-values and summary statistics see Table 3.1). However, variegated fairy-wrens did alter their behavior (Z-transform test Z = -3.25, $P \leq 0.001$, for independent test p-values and summary

statistics see Table 3.1), spending a greater proportion of time foraging when in the presence of splendid fairy-wrens (Table 3.1; mean = 0.76 ± 0.03, S.E.) than when solitary (mean = 0.66 ± 0.04, S.E., paired *t*-test = 2.31, *P* = 0.04) and less time traveling in the presence of splendid fairy-wrens (Table 3.1; mean = 0.03 ± 0.01, S.E.) than when solitary (mean = 0.06 ± 0.02, S.E., paired *t*-test = 3.01, *P* = 0.01).

Table 3.1. Results of statistical tests of behavior when birds were associating with their co-resident and when not. The categories foraging, singing, traveling and contact calling were analyzed with a paired-samples *t*-test while the category mobbing was analyzed with a Friedman test and the category vigilance was analyzed with a related samples Wilcoxon signed rank test. Sample size is 11 for all splendid categories and 12 for all variegated categories. Contact calling was square-root transformed, all other categories are non-transformed. * indicates $p \leq 0.05$.

		Mean associating ± S. E.	Mean solitary ± S. E.	Test statistic	p-value
Splendid fairy-wren	Foraging	0.64 ± 0.04	0.56 ± 0.05	1.41	0.19
	Singing	0.25 ± 0.04	0.25 ± 0.04	0.03	0.98
	Traveling	0.07 ± 0.06	0.09 ± 0.02	-1.11	0.29
	Contact calling	0.13 ± 0.04	0.21 ± 0.06	-1.97	0.08
	Mobbing	0.00 ± 0.00	0.01 ±0.01	3.00	0.23
	Vigilance	0.24 ± 0.05	0.22 ±0.03	25.00	0.48
Variegated fairy-wren	Foraging	0.76 ± 0.03	0.66 ± 0.04	2.31	0.04*
	Singing	0.14 ± 0.02	0.19 ± 0.02	-1.52	0.16
	Traveling	0.03 ±0.01	0.06 ± 0.02	-3.01	0.01*
	Contact calling	0.19 ± 0.04	0.24 ± 0.05	-0.74	0.49
	Mobbing	0.01 ± 0.00	0.00 ± 0.00	4.00	0.59
	Vigilance	0.06 ± 0.01	0.14 ± 0.01	78.00	0.002

Variegated fairy-wren groups were less vigilant than splendid fairy-wren groups (10 and 22% of the observed time on average, respectively, *t*-test = 3.95, df = 21, *P* = 0.001). As in the

case of foraging and traveling, splendid fairy-wrens did not change their vigilance behavior when in the presence of the heterospecific (Related-samples Wilcoxon signed rank test $Z = 25.00$, $P = 0.48$), but variegated fairy-wrens did change, reducing their vigilance (Wilcoxon signed rank test $Z = 78.00$, $P = 0.002$, Figure 3.2, see Table 3.1 for descriptive statistics).

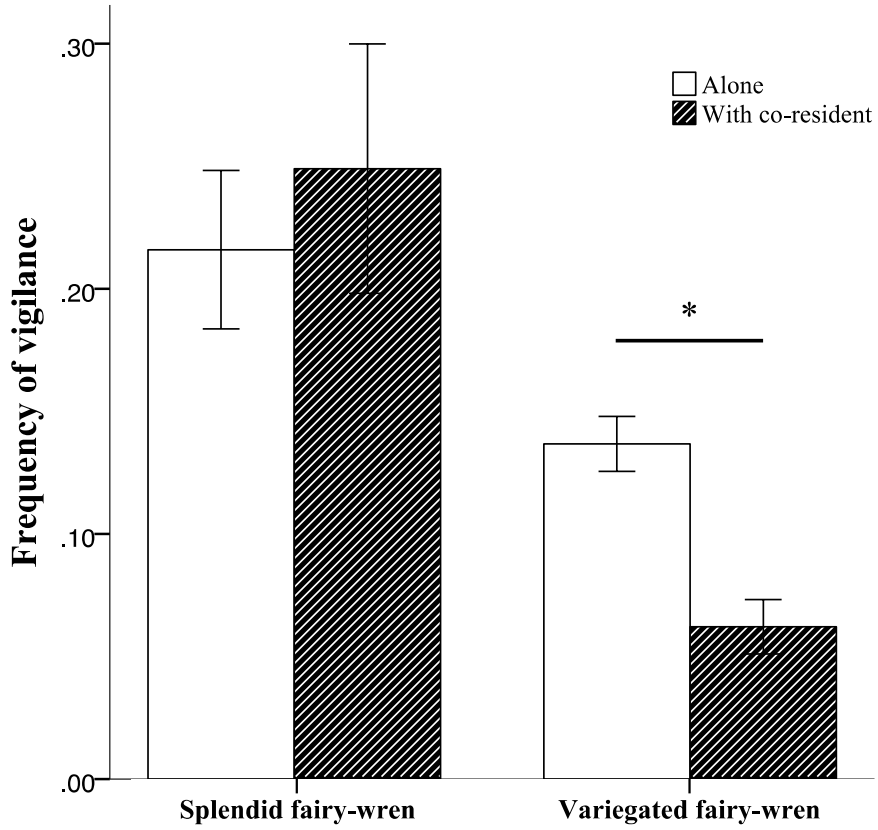

Figure 3.2. Frequency of vigilance behavior in splendid and variegated fairy-wrens in co-incident territories in the presence (solitary) and absence (with co-resident) of their heterospecific co-resident, grouped by species. Solitary territories are indicated by clear bars, shared territories by hashed bars. * indicates $P < 0.05$. Analyses are separate for each species. Analyses are separate for each species. Error bars indicate ± 1 standard error.

Discrimination among heterospecific songs

Splendid fairy-wrens responded more strongly to the songs of neighboring and foreign variegated fairy-wrens than to either the co-resident's songs or control songs (repeated measures ANOVA, $P < 0.001$, Figure 3.3A; see Figure 3.4 for an example response to stimuli; see Table 3.2 for pairwise *P*-values and Table 3.C.1 for descriptive statistics). As expected, there was no significant difference between the number of Type II songs produced by splendid fairy-wrens in response to any treatment category (Friedman's Test, $P = 0.819$). Variegated fairy-wrens responded more strongly to the songs of neighboring and foreign splendid fairy-wrens than to either the co-resident's songs or to the control songs (repeated measures ANOVA, $P < 0.001$, Figure 3.3B for PCA response values; see Table 3.2 for pairwise p-values and Table 3.C.2 for descriptive statistics).

Conspecific neighbor-stranger discrimination

Splendid fairy-wrens responded more strongly to neighboring and foreign conspecific songs than to control songs, and again, showed no difference in their response to neighboring or foreign conspecific songs (repeated measures ANOVA $P \leq 0.001$, Figure 3.5A; see Table 3.3 for pairwise p-values and Table 3.C.3 for descriptive statistics). As before, there was no significant difference between the number of Type II songs produced by splendid fairy-wrens in response to any treatment category (Friedman's test $P = 0.331$). Similarly, variegated fairy-wrens responded more strongly to neighboring and foreign conspecific songs than to control songs, however there was no difference in their response to neighboring or foreign conspecific songs (repeated

measures ANOVA $P \leq 0.001$, Figure 3.5B; see Table 3.3 for pairwise p-values and Table 3.C.4 for descriptive statistics).

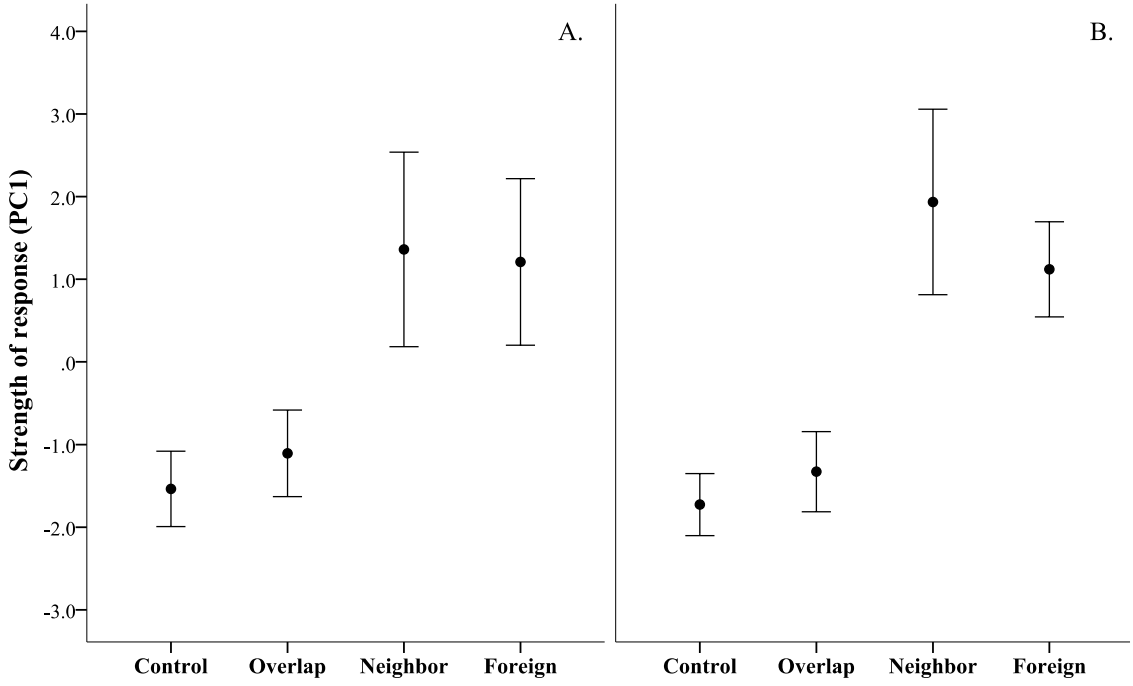

Figure 3.3. The principle component score measure of male A.) splendid and B.) variegated fairy-wren responses to heterospecific songs as derived from the five aggressive response variables. Treatment categories are songs of a 1) control, the red-capped robin, 2) co-resident heterospecific fairy-wren, 3) neighboring heterospecific fairy-wren, and 4) foreign heterospecific fairy-wren. Pairwise comparisons between control-neighbor, control-foreign, co-resident-neighbor, and co-resident-foreign are significant ($P < 0.05$) for both species. Error bars indicate 95% confidence intervals.

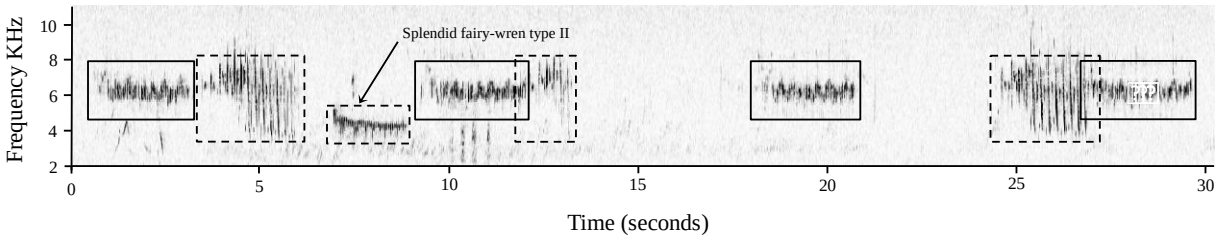

Figure 3.4. Spectrogram illustrating an example of a splendid fairy-wren's response to a variegated fairy-wren intruder treatment. Solid boxes indicate variegated fairy-wren Type I song stimulus and dashed boxes indicate splendid fairy-wren singing response. All responses in this example are Type I songs except for one Type II that is indicated in the figure.

Table 3.2. Pairwise comparisons of heterospecific playback treatment response (principle component scores) of each species (repeated measures ANOVA). * indicates $p \leq 0.05$.

		Co-resident	Neighbor	Foreign
Splendid fairy-wren	Control	1.000	0.001*	0.001*
	Co-resident		0.017*	0.008*
	Neighbor			1.000
Variegated fairy-wren	Control	1.000	≤0.001*	≤0.001*
	Co-resident		≤0.001*	0.001*
	Neighbor			1.000

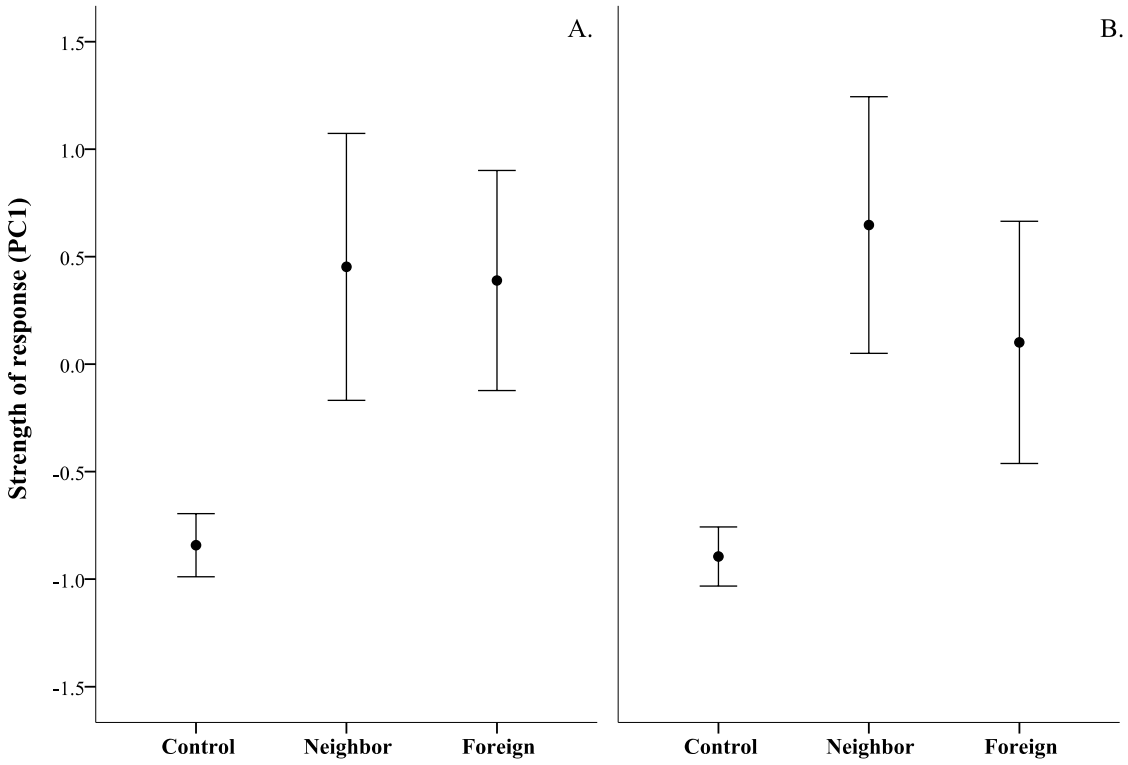

Figure 3.5. The component score measure of male A.) splendid and B.) variegated fairy-wrens to consepecific neighbor and stranger songs as derived from the seven conspecific aggressive and mate-guarding response variables. Treatment categories are songs of a 1) control, the red-capped robin, 2) neighboring conspecific male and 3) foreign conspecific male. Pairwise comparisons between control-neighbor and control-foreign are significant ($P < 0.05$) for both species. Error bars indicate 95% confidence intervals.

Table 3.3. Pairwise comparisons of conspecific playback treatment response (principle component scores) of each species (Friedman's test). * indicates p≤0.05.

		Neighbor	Foreign
Splendid fairy-wren	Control	0.001*	0.002*
	Neighbor		1.00
Variegated fairy-wren	Control	0.004*	≤ 0.001*
	Neighbor		0.499

DISCUSSION

Both variegated and splendid fairy-wrens generally tolerate the other species when they share a common territory, but act aggressively towards the other species when they do not. Why, then, is the presence of certain heterospecific individuals tolerated while the presence of other individuals is not? It is possible that shared territories are only observed when birds are unable to effectively repel the other species, either because shared territories are in high quality habitat so competition is high or alternatively, because birds on shared territories are lower quality. Birds may accept the presence of a heterospecific co-resident and become habituated to their song, but attempt to defend their territory from any additional heterospecific intrusions. However, decrease in vigilance behavior by variegated fairy-wrens in the presence of splendid fairy-wrens is likely not habitat specific and represents a benefit of associating. Anecdotally, variegated fairy-wrens have been observed to join their co-residents and actively help in defending the area against intruders or mobbing predators (Johnson, personal observation). While increased re-nesting in shared territories may also be indicative of habitat quality, our data suggests there is a clear benefit to variegated fairy-wrens of sharing a territory with splendid fairy-wrens.

Sharing a territory may benefit both species through information sharing related to food resources, and by an increased effective group size without increased competition that fosters a cooperative association. This would be particularly beneficial in terms of reducing the risk of predation and increasing the foraging space used by each species (as both species exhibit different foraging behaviors when alone, Tibbetts and Pruett-Jones 1999). While behavioral changes in foraging, nesting and vigilance were only observed in variegated fairy-wrens, we believe that splendid fairy-wrens also likely benefit. If this is the case, discrimination between co-resident and non-co-resident heterospecifics may arise for two reasons. In order to gain the

benefits of cooperating with the other species on their territory, birds may have to be willing to exclude heterospecifics outside of the territory in a sort of interspecific reciprocal cooperation (e.g. "pay-to-stay" hypothesis in cooperatively breeding species; Gaston 1978, Kokko et al. 2002). From the perspective of an individual bird, a splendid fairy-wren may only tolerate a variegated fairy-wren that it shares a territory with if that variegated fairy-wren helps it exclude other splendid fairy-wrens. The mating system of most fairy-wren species is characterized by extreme reproductive promiscuity (Webster et al. 2004, Cockburn et al. 2013), and extra-pair matings can come from neighboring or foreign males, such that neighbors represent as great a threat as foreign individuals. This does seem to be the case, in fact, as we do not observe the dear-enemy effect within species in either variegated for splendid fairy-wrens. This may also explain why the dear-enemy effect is not observed across species either. Alternatively, associating with heterospecifics may be beneficial, but a stable relationship may be more beneficial than a new relationship. If experience has a positive effect on the group's ability to move together or coordinate behaviors, maintaining relationships with known heterospecifics rather than negotiating new relationships with unfamiliar individuals may be in the group's best interest. More research will need to be performed to distinguish between these possibilities.

Finally, variegated fairy-wrens appear to benefit more than splendid fairy-wrens from this interspecific association. Interacting socially with heterospecifics increases effective group size, and one possible benefit of such interactions is reduced individual vigilance (Pulliam 1973), which we observe in variegated fairy-wrens associating with splendid fairy-wrens. Variegated fairy-wrens also increased their first nest fledging success and were more likely to re-nest following nest failure when on territories shared with splendid fairy-wrens. Variegated fairy-wrens generally have larger social groups than splendid fairy-wrens (Tideman 1986, Tibbetts and

Pruett-Jones 1999), and large social groups facilitate the successful fledging of young in variegated fairy-wrens, with groups with auxiliary member successfully fledging young more frequently than solitary pairs (Johnson and Pruett-Jones, in prep). The two species also partition the foraging habitat, with splendid fairy-wrens foraging primarily on the ground and variegated fairy-wrens foraging in small bushes or mallee trees (Tibbetts and Pruett-Jones 1999). Dissimilar resource use and reduced competition in heterospecific interactions relative to conspecific interactions has been hypothesized to explain some examples of mutualisms and commensalisms (Seppäen et al. 2007, Sridhar et al. 2009). We hypothesize that associations with the splendid fairy-wren allow the variegated fairy-wrens to increase functional group size, effectively increasing the social group size to include conspecific and heterospecific individuals without incurring the competitive cost of additional conspecific helpers.

Variegated and splendid fairy-wrens are non-migratory and permanently resident in their habitat. As such, heterospecific associations have the opportunity to persist across years and may have long-term beneficial consequences. Beneficial heterospecific associations have long been recognized in birds, from brief mixed species foraging flocks, to stable co-defended foraging territories observed in the tropics (Munn and Terborgh 1979, Sëppanen et al. 2005). The evidence for heterospecific beneficial association mediated by song recognition is novel. Cooperative associations between individuals of different species for the purposes of foraging, predator detection, predator deterring, and information sharing are common in many other systems as well, from cooperative associations between eels and groupers for hunting to honeyguides and humans for the excavation of beehives (Isack and Reyer 1989, Bashary et al. 2006, Magrath et al. 2009, Dinets and Eligulashivili 2016). Other long-term cooperative associations between heterospecific species and even antagonistic or territorial interactions may

similarly be maintained by recognition and discrimination between known and unknown individuals. Heterospecific associations have the ability to alter species ranges, species recruitment, breeding success, and even behavioral specialization (Powell 1989, Mönkkönen et al. 1996, Sëppanen et al. 2007, Goodale et al. 2010). Such long-term associations mediated by recognition may have unique consequences for the behavior and ecology of both species.

APPENDIX 3A: LOADINGS DERIVED FROM PCA ON RESPONSE VARIABLES FOR HETEROSPECIFIC AND CONSPECIFIC RECONGITION EXPERIMENTS

Table 3.A.1. Loadings derived from PCA of the five aggressive response variables. Analysis was done on variegated and splendid fairy-wren responses separately to account for species-specific behavior. Splendid fairy-wren responses loaded onto two factors, however all behavioral responses loaded strongly onto PC1, which was used in subsequent analyses.

Response variable	Splendid fairy-wren PC1	Splendid fairy-wren PC2	Variegated fairy-wren PC1
Latency of response	-0.77		-0.76
Approach	0.87		0.69
Songs (Type I and Type I+II)	0.74		0.81
Duration of vigilance	0.38	0.87	0.69
Scolds	0.55	-0.47	0.71

Table 3.A.2. Loadings derived from PCA of the aggressive and mate guarding response variables recorded during the conspecific neighbor-stranger experiments. Analysis was done on variegated and splendid fairy-wren responses separately to account for species-specific behavior. Splendid fairy-wren responses loaded onto a single factor. Variegated fairy-wren responses loaded onto two factors, however all behavioral responses loaded strongly onto the PC1, which was used in subsequent analyses.

Response variable	Variegated fairy-wren PC1	Variegated fairy-wren PC2	Splendid fairy-wren PC1
Latency of response	-0.66		-0.52
Approach	0.85		0.80
Bill-wipe	0.49	0.62	0.70
Mate-guarding	0.57	-0.56	0.63
Look	0.30	0.68	0.66
Songs (Type I and Type I+II)	0.70		0.55
Duration of vigilance	0.77		0.89

APPENDIX 3B: CROSS TABULATION OF SPLENDID AND VARIEGATED NESTING SUCCESS AND RE-NESTING BEHAVIOR ON SHARED AND SOLITARY TERRITORIES

Table 3.B.1. Cross tabulation table of number of groups fledging offspring from their first nest, fledging any young, and attempting to re-nest following initial nest failure of both splendid and variegated fairy-wrens. All comparisons have one degree of freedom.

		Solitary territory	Shared territory	X^2	p-value
Splendid fairy-wren	Fledged first nest	10	27	1.60	0.14
	Did not fledge first nest	45	72		
	Fledged	14	33	2.64	0.10
	Did not fledge	43	55		
	Re-nested	25	36	0.13	0.72
	Did not re-nest	25	41		
Variegated fairy-wren	Fledged first nest	6	30	5.97	0.02
	Did not fledge first nest	35	54		
	Fledged	19	35	0.01	0.92
	Did not fledge	32	61		
	Re-nested	5	32	6.99	<0.01
	Did not renest	28	46		

APPENDIX 3C: RAW RESPONSES OF SPLENDID AND VARIEGATED FAIRY-WRENS TO HETEROSPECIFIC AND CONSPECIFIC PLAYBACK EXPERIMENTS

Table 3.C.1. Descriptive statistics of splendid fairy-wren raw responses to heterospecific playback stimuli.

	Control	Co-resident	Neighbor	Foreign
Latency to response (sec)				
Mean	720.07	513.36	175.07	84.53
Median	1080.00	295.50	29.00	27
Std. Error	128.84	124.38	83.30	32.06
Approach				
Mean	0.07	0.00	1.71	1.47
Median	0.00	0.00	2.00	1.00
Std. Error	0.07	0.00	0.29	0.42
Songs (Type I and Type I+II)	0.73	1.64	5.07	5.00
Mean	0.00	0.5	4.00	3.00
Median	0.56	0.98	1.13	1.41
Std. Error				
Duration of vigilance (sec)				
Mean	0.00	6.29	7.92	7.47
Median	0.00	0.00	10.00	0.00
Std. Error	0.00	4.01	13.84	5.58
Scold				
Mean	0.20	0.14	12.21	7.33
Median	0.00	0.00	4.00	3.00
Std. Error	0.14	0.14	7.13	2.51

Table 3.C.2. Descriptive statistics of variegated fairy-wren responses to heterospecific playback stimuli.

	Control	Co-resident	Neighbor	Foreign
Latency to response (sec)				
Mean	564.73	452.80	14.07	22.97
Median	457.00	380.00	12.00	18.00
Std. Error	118.06	104.27	2.65	5.55
Approach				
Mean	0.00	0.33	1.47	1.26
Median	0.00	0.00	1.00	1.00
Std. Error	0.00	0.16	0.29	0.21
Songs (Type I and Type I+II)				
	0.60	2.00	6.87	5.20
Mean	0.00	1.00	3.00	5.00
Median	0.34	0.82	1.59	1.22
Std. Error				
Duration of vigilance (sec)				
Mean	0.00	0.00	38.20	16.60
Median	0.00	0.00	0.00	0.00
Std. Error	0.00	0.00	12.58	9.09
Scold				
Mean	1.27	0.27	14.80	11.27
Median	0.00	0.00	11.00	7.00
Std. Error	1.02	0.18	3.71	3.87

Table 3.C.3. Descriptive statistics of splendid fairy-wren raw responses to conspecific playback stimuli.

		Control	Neighbor	Foreign
Latency of response				
	Mean	460.90	77.18	21.36
	Median	390.72	30.73	20.91
	Std. Error	96.83	31.80	5.07
Approach				
	Mean	0.07	3.21	2.64
	Median	0.00	2.00	2.50
	Std. Error	0.07	0.64	0.60
Bill-wipe				
	Mean	0.00	0.21	0.14
	Median	0.00	0.00	0.00
	Std. Error	0.00	0.11	0.10
Mate-guarding				
	Mean	0.21	0.93	1.86
	Median	0.00	0.00	1.00
	Std. Error	0.11	0.40	0.66
Look				
	Mean	0.00	1.00	0.50
	Median	0.00	0.00	0.00
	Std. Error	0.00	0.42	0.23
Songs (Type I and Type				
I+II)		2.79	6.36	10.43
	Mean	0.50	2.50	9.50
	Median	1.11	1.99	1.89
	Std. Error			
Duration of vigilance				
	Mean	37.51	219.12	225.93
	Median	22.82	125.06	133.96
	Std. Error	11.75	48.70	64.79

Table 3.C.4. Descriptive statistics of variegated fairy-wren responses to conspecific playback stimuli.

		Control	Neighbor	Foreign
Latency of response				
	Mean	381.40	132.7451	58.45
	Median	223.31	31.06	16.41
	Std. Error	98.67	55.79	20.21
Approach				
	Mean	0.00	2.54	3.57
	Median	0.00	2.00	4.00
	Std. Error	0.00	0.53	.488
Bill-wipe				
	Mean	0.00	0.23	0.14
	Median	0.00	0.00	0.00
	Std. Error	0.00	0.12	0.10
Mate-guarding				
	Mean	0.00	0.54	0.86
	Median	0.00	0.00	0.00
	Std. Error	0.00	0.24	0.345
Look				
	Mean	0.00	0.46	0.50
	Median	0.00	0.00	0.00
	Std. Error	0.00	0.24	0.31
Songs (Type I and Type				
I+II)		2.50	4.69	7.64
	Mean	1.00	5.00	8.00
	Median	1.32	0.84	1.22
	Std. Error			
Duration of vigilance				
	Mean	1.46	79.78	171.49
	Median	0.00	46.35	187.14
	Std. Error	1.03	35.94	35.61

CHAPTER 4: HELPING BEHAVIOR AND PROMISCUITY IN THE VARIEGATED FAIRY-WREN: SEX SPECIFIC EFFECTS OF AUXILIARY MEMBERS

ABSTRACT

Cooperative breeding is a social system in which auxiliary group members assist in the rearing of non-descendant young. If and how the addition of auxiliary group members contributes to nesting success have been the focus of much work. However, little work has been done to examine the potential importance of auxiliary member sex. We examine the role of both auxiliary number and sex on several aspects of reproduction in the variegated fairy-wren (*Malurus lamberti*). The probability of fledging young and egg volume both positively correlated with the number of male auxiliaries, but not females. Dominant males also decrease their provisioning rate as the number of male auxiliaries increased, a pattern that was not observed in dominant females or with number of female auxiliaries. This compliments recent work done in the related red-backed fairy-wren (*M. elegans*) that demonstrates the importance of social context on helping behavior. We similarly found an importance of social context, with the presence of male, but not female auxiliaries correlating with increased egg volume and load lightening of dominant male parental previsioning rates. We found that extra-pair paternity rate decreased in groups with more male, but not female, auxiliaries, a relationship that is opposite of the pattern in other fairy-wren species. The number of both male and female auxiliaries correlated with fledging success. These benefits may explain why such large group sizes are tolerated by dominant males in this species.

INTRODUCTION

Explaining diversity in animal societies has remained a challenge for evolutionary biology despite much empirical and theoretical work. Cooperative breeding, a social system typically characterized by alloparenting, occurs when offspring of one or both sexes delay their dispersal and remain with their social parents in successive generations, subsequently assisting the breeding pair in raising additional offspring. It can also occur when unrelated individuals join social groups and assist the dominant pair in raising young (Brown 1987). Explaining the evolution of cooperative breeding as a social system is particularly difficult because in many cases, auxiliary individuals exhibit ostensibly altruistic behavior in the raising of non-descendant young while there is a dearth of evidence for this 'helping' behavior in other systems (Cockburn 2006).

Auxiliaries are predicted to remain on their natal territory or join groups when 1) the habitat is saturated and breeding vacancies do not exist ("ecological constraints," Emlen 1982), 2) when group care is obligatory (as in the white-winged chough, Heinsohn 1992), or 3) when there are intrinsic benefits to delayed dispersal or group living (Woolfenden and Fitzpatrick 1978, Koenig et al. 1992). While the presence of helpers results in an increase in survival of young in some species (Brown 1987, Stacey and Koenig 1990), in many cases the benefits of auxiliaries consist of load lightening, increased provisioning rate, and increased survival of the breeding female or male (Tidemann et al. 1986, Wright and Dingemanse 1999, Heinsohn 2004, Russell et al. 2007, Spong et al. 2008). Across species, however, auxiliaries do not always "help" and they may not have any effect on survival of offspring (Magrath and Yezerinac 1997, Cockburn 1998). Auxiliaries may even compete with dominant members for resources (Brouwer et al. 2006), mates (Double and Cockburn 2002), or care within systems where reproduction is

shared across individuals (Burke et al. 1989, Whittingham et al. 1997, Richardson et al. 2001). Thus the benefits for breeding individuals who permit auxiliaries to remain on a territory have continued to be the focus of much research. Most avenues of research have scrutinized the benefits of helping behavior specifically in terms of overall group size, or presence or absence of auxiliaries, whereas less work has been done to address the potential importance of auxiliary member sex (Brouwer et al. 2014).

Social dynamics and social context are likely important in the distribution of helping behaviors. Depending on the system, individuals of a particular status (breeder or helper) or of a particular sex may benefit more from assisting. For example, if female auxiliaries gain experience that contributes to their reproductive success later in life, females may be more likely to provide care than males. Alternatively, if males are likely to inherit a territory, they may assist more to increase the likelihood they are allowed stay ("pay-to-stay," Clutton-Brock 2002). Presence of helpers has additionally been shown to influence extra-pair paternity rates in the small subset of cooperatively-breeding species that exhibit high rates of extra-pair paternity (Mulder et al. 1994, Webster et al. 2004). In these species, the fairy-wrens, helpers appear to release females to engage in extra-pair mating by mitigating the potential for male retaliation or withholding of parental care (Mulder et al. 1994). While previously not addressed, social context may also strongly influence rates of extra-pair paternity if auxiliaries of different sexes pose different threats to dominant male paternity (especially when helpers are unrelated), or if the auxiliary members present do not provide help and thus do not release females from possible male retaliation.

Variation in auxiliary sex, auxiliary number, and the incidence of cooperative breeding within and between populations in the same habitat (e.g., Tidemann 1988, 2004, Tibbets and

Pruett-Jones 1999), as well as across species, suggests that the costs and benefits of cooperative breeding are mutable, and may be highly influenced by context. For example, in the red-winged fairy-wren (*Malurus elegans*) auxiliary group member sex has implications for parental care and the fitness of nestlings later in life (Brouwer et al. 2014), with the presence of female helpers benefiting young more than the presence of males. The authors propose the "social environment" hypothesis of investment, which emphasizes that not only the number, but also the sex and type of auxiliary group members is crucial in determining investment from the rest of the social group (Brouwer et al. 2014).

Much of the best data regarding cooperative breeding comes from studies in birds. While helping behavior and promiscuity have been examined in many cooperatively breeding bird species (Woolfenden and Fitzpatrick 1984, Brown 1987, Stacy and Ligon 1991, Burt and Peterson 1993, Mulder et al. 1994, Komdeur 1992, Wright and Dingemanse 1999), most of the species studied to date exhibit rather little variation in either or both the identity or number of helpers. Here we examine helping behavior and promiscuity in the variegated fairy-wren (*Malurus lamberti*), specifically to examine both the effect of multiple helpers on behavior and nesting success, as well as the effect of social group composition on these behaviors. The variegated fairy-wren is an ideal species for the study of group composition in helping behavior, as group size can range from 2-10 birds, and both males and females are common as auxiliaries. As the effects of "helpers" take many forms across systems, we consider several ways helpers may contribute to group success or dynamics. First, we examine the role of group size and auxiliary sex on fledging success. Then we examine the role of group size and auxiliary sex on egg size, nestling weight, and provisioning rate. Finally, the presence of helpers in other cooperative breeding systems is theorized to release females from a potential loss of paternal

care from her social mate if she engages in extra-pair matings. This results in a pattern of increased extra-pair paternity as group size increases (Mulder et al. 1994, Webster et al. 2004). Because large numbers of auxiliary group members are tolerated in variegated fairy-wrens, we examined rates of extra-pair paternity to determine if this pattern persists in this system and if there is variation with auxiliary sex.

METHODS

Study system

All species in *Malurus* are known or suspected to exhibit cooperative breeding (Russell and Rowley 2007). While cooperative breeding is pervasive in this clade, there is considerable variation across species in aspects of ecology and social organization, including group size and the sex of auxiliary members. For this reason, fairy-wrens have become model systems for the study of the costs, benefits, and drivers of cooperative breeding. The variegated fairy-wren is ideal for examining the role of both group size and group composition in nesting success and extra-pair paternity, as across groups within a population and across populations it exhibits variation in almost all aspects of social organization.

Observational field methods and collection of DNA and morphological measurements

This research was conducted at Brookfield Conservation Park (BCP) from late September to mid-December each year from 2012-2015. BCP, located in South Australia (S 34°21', E 139°29'), is characterized by mallee eucalyptus and chenopod scrub habitat and has populations of three species of fairy-wrens, the variegated, splendid (*M. splendens*), and white-winged fairy-wren (*M. leucopterus*).

A color-banded population of variegated fairy-wrens was established at this site beginning in 2012. Variegated fairy-wrens were historically studied and color-banded at BCP from 1992-1999, after which banding and monitoring ceased. While variegated fairy-wrens were banded intermittently at the site starting in 2004, the population was essentially unmarked when we began extensively monitoring it in 2012.

Adult birds were captured by targeted mist netting, a method which minimizes bi-catch and the duration of time birds were left alone in the net unattended. Adults were banded both with a unique combination of three color-bands and an individually numbered metal band issued by the Australian Bird and Bat Banding Scheme. A blood sample was taken from each bird by brachial vein puncture and stored dry on Whatman® FTA cards. Each year between 36 and 71 family groups were studied. Family groups contained at least one male and one female, however groups often contained auxiliary group members, with the largest social group containing 7 auxiliaries. Mean group size ranged from 2.91 to 4.24 with an average group size of 3.30 ± 1.41 SD (n = 220). Across years, 63.63% of groups contained one or more auxiliary members.

Once groups were identified, group composition, nesting behavior and territory size were monitored. Variegated fairy-wrens are highly social and group members were often found together. Rarely were birds seen outside their social territory by themselves and in most cases these were auxiliary females. Social status of males and females was determined by behavior, as well as known age and plumage variation. As with other fairy-wren species (Mulder et al. 1994, Webster et al. 2004, Varian-Ramos and Webster 2012, Magraf and Cockburn 2013), many auxiliary group members in our population of variegated fairy-wrens were sons or daughters of one or both members of a breeding pair from previous years. Plural breeding, in which an auxiliary female also nests, was observed in a small number of groups, an observation consistent

with other species (Rowely et al. 1989, Brouwer et al. 2011). Between 2012 and 2015 5% of territories exhibited plural breeding (11 of 220 social groups). Each time birds were encountered or followed their location was recorded with a GPS unit, with the outer limits of these observations denoting approximate territory boundaries.

Once nests were located, they were followed through to failure or fledging. If nests failed (the nest was depredated, abandoned, or the chicks died) the group (and female) was monitored for re-nesting behavior. If nests successfully produced nestlings, each chick was weighed at 3 and 6 days old (DO). The weights of nestlings were taken between 0800 and 1100 AM. Blood samples were collected from chicks when they were between 3 and 8 DO. Nestlings that survived to 6 DO were banded with a metal band. In 2014 and 2015 egg volume was also measured. Egg length and egg width were measured with digital calipers then used to calculated egg volume (length × maximum width ^{2} × $\pi/6$, Hoyt 1979). Eggs were measured from 72 clutches, representing reproductive attempts of 57 females.

While some breeding occurs at this site into January, few groups initiated nests after the beginning of December and our sampling represents the majority of offspring produced by the population.

Helping behavior and group composition

To examine the role of group size and group composition on parental care nest, provisioning of nestlings was monitored at 36 nests of dominant breeding females in groups of varying size from 2012-2014. We attempted to observe nests for 2 hours between 0800 and 1130 AM for three mornings when chicks were 5-7 DO. Not all nests were observed for the entire duration due to extenuating circumstances (i.e. depredation, weather). Nests were observed for

an average total of 295.22 min ± 96.49 SD. Total number of feeding visits, the identity of the bird performing each feeding event, and duration of vigilance behavior at or near the nest was recorded. Provisioning rate for the entire group and each individual was averaged across all days of observation.

Genotyping and determination of paternity

We quantified rates of extra-pair paternity for groups studied from 2012-2014 we estimated rates of extra-pair paternity. Only nests in which all group members were sampled were included in our analyses. Each year as much of the population as possible was genotyped to accurately assess allele frequency and to include the majority of males in paternity analyses. A total of 238 individuals in 2012, 257 in 2013, and 334 in 2014 were included in the analyses, with nestlings genotyped from 23, 39, and 49 nests in 2012, 2013 and 2014 respectively.

DNA was extracted using a modified version of Qiagen's DNeasy Blood and Tissue Kit protocol for extraction of DNA from whole blood stored on FTA cards. Individuals were genotyped using 6 highly polymorphic microsatellite loci developed for related fairy-wren species and optimized for use in the variegated fairy-wren. Each locus was amplified using fluorescently-labeled primers and a standard PCR protocol. Loci, optimized annealing temperature, and original species each loci was developed for can be found in Table 4.1. Samples were genotyped by the University of Chicago DNA Sequencing & Genotyping Facility then sized using Peak Scanner™ 1.0 (Applied Biosystems). Raw peak sizes were then binned to best fit the expected base pair repeat described for each locus. All loci approximately matched the expected size, except for *Mcyu 8*, which switched from a tetranucleotide repeat to a dinucleotide

repeat at one site then resumed a shifted tetranucleotide peak. Because this occurred in only one site and the same peaks were observed across many individuals, the binning was accepted.

Table 4.1. Original isolation of loci used for variegated fairy-wren genotyping and the optimized annealing temperatures.

Locus	Optimized annealing temperature	Species developed in	Citation
Mcyu2	62	*M. cyaneus*	Double et al. 1997
Msp10	65	*M. splendens*	Webster et al. 2004
Smm7	54	*Stipiturus malachurus*	Maguire et al. 2006
Mcyu7	62	*M. cyaneus*	Double et al. 1997
Mcyu8	62	*M. cyaneus*	Double et al.1997
Mcyu3	55	*M. cyaneus*	Double et al. 1997

Cervus 3.0 (Mashall et al. 1998, Kalinowski et al. 2007) was used to assess allele frequencies, estimate expected and observed heterozygosity, null allele frequency, and assign paternity. Analyses were completed for each year independently. Three loci, *Mcyu 2*, *Mcyu8*, and *Mcyu3*, deviated significantly from Hardy-Weinberg equilibrium ($P < 0.05$) consistently across all three years, suggesting the presence of null alleles (Pemberton et al. 1995). The presence of null alleles can influence mismatches between known parent-offspring pairs resulting in typing errors. Cervus estimates null allele frequencies using the method described by Summers and Amos (1997). Small null allele frequencies (< 0.05) generally do not affect error rate. Two loci used in our analyses exhibit high null allele frequencies (*Mcyu 2* and *Mcyu3*), however, both loci were left in analyses because they exhibited a low genotyping error rate (Table 4.2). A maternal exclusion probability and a paternal exclusion probability were calculated for each locus. Maternal exclusion probability is the probability that a randomly selected candidate mother will not match the nestling at a given locus when no parental genotype is known. Across all years

combined maternal exclusion probability was nearly identical at ~ 0.99. Paternal exclusion probability is the probability that a randomly selected candidate father will be excluded assuming that maternal genotype is known. Combined paternal exclusion was approximately the same across all years at ~0.99.

Table 4.2. Variability of microsatellite loci used in paternity and relatedness analyses, based on 2012 samples (*n* = 238 individuals, 218-236 individuals typed per loci).

Locus	No. alleles	Heterozygosity		Maternal exclusion probability	Paternal exclusion probability	Null allele frequency	Genotyping error rate
		Observed	Expected				
Mcyu2	9	0.322*	0.594	0.184	0.315	0.303	0.000
Msp10	17	0.835	0.834	0.524	0.691	-0.004	0.000
Smm7	19	0.886	0.887	0.626	0.771	-0.002	0.014
Mcyu7	11	0.591	0.640	0.228	0.383	0.045	0.000
Mcyu8	39	0.900*	0.952	0.818	0.900	0.027	0.011
Mcyu3	26	0.601*	0.932	0.751	0.858	0.215	0.025
Combined				0.994	0.999		0.008

* Significantly deviates from Hardy-Weinberg equilibrium; goodness-of-fit tests, df=1, $P < 0.05$.
Maternal exclusion probability is the probability that a randomly selected candidate parent will not match the chick at a given locus when no parent genotype is known. Paternal exclusion probability is the probability that a randomly selected candidate father will be excluded assuming the maternal genotype is known.

Cervus was also used to carry out paternity analysis. Because egg laying by individual females was often observed, and there were no instances of two females incubating the same clutch, we were highly confident in maternal identity. Cervus calculated a likelihood score (LOD score) for all males in the population given the genotype assigned for the mother, confidence of the LOD score of the candidate father, as well as of the trio (offspring, mother, and father). Cervus assigned fathers to an average of 87% of offspring at a relaxed confidence of 80% across years. In some cases, Cervus did not assign confidence in the assignment of any male. In these

cases, the male with the highest LOD score was accepted as the father if the male was the social father and had fewer than 2 mismatches. In general, the male assigned by Cervus with the highest LOD score was accepted as the father, at a rate of 86%. We did not use the assignment made by Cervus when a lower ranked male had a similar LOD score but fewer mismatches or if a lower ranked male had a similar LOD score but was the social father. In the latter case, the social father was kept in favor of the male with the highest LOD score only if both males had the same number of mismatches. In most cases when the social male was accepted over the male with the highest LOD score the trio confidence was significant for the social male. While the loci used had a high exclusion probability, they may still misidentify potential fathers, particularly when candidate fathers are related (Double et al. 1997), and these exceptions were designed to improve the accuracy of paternity assignment.

While paternity was assigned with confidence for most chicks, for the purposes of the analyses carried out in this chapter we were only interested in whether each chick was the product of the social father or of an extra-pair mating. Therefore, in cases when no male was supported with a high confidence and the social male had a low LOD score, the chick was identified as extra-pair (not sired by the social father) even though paternity was not assigned. One nest was excluded from paternity analysis because 3 or more mismatches occurred between the mother and one or more of her chicks, suggesting either misidentification of the mother or genotyping error.

Statistical analysis

All statistical analyses were conducted in JMP® 12.2.0. Fledging success was fitted to a nominal logistic model with group size, number of male auxiliaries and number of female auxiliaries as parameters. Analysis of fledging success did not take into account the number of

nests attempted by the breeder (renesting only occurred following initial nest failure), but was scored as the presence or absence of fledglings at any point during the season. Groups that had chicks at the end of the breeding season but had not yet fledged when we left the field site were excluded from the analysis of fledging success. Mean egg volume, mean chick weight at 3 DO, and mean chick weight at 6 DO were fit to separate standard least squares models with group size, number of male auxiliaries, number of female auxiliaries, and Julian date of incubation initiation as parameters. Clutch size was also included as a parameter for the mean egg volume model. Number of chicks at 3 DO and number of chicks at 6 DO were included for models of mean chick weight at 3 DO and 6 DO respectively.

Total provisioning rate was fitted as a response variable in a standard least square model with the parameters group size, number of male auxiliaries, and number of female auxiliaries. Dominant male and dominant female provisioning were also fitted as response variables in standard least squares models with the parameters group size, number of male auxiliaries, number of female auxiliaries, and dominant male provisioning rate.

We used nominal logistic models to examine whether the probability that a clutch contained at least one extra-pair young changed with number of male auxiliaries and number of female auxiliaries. We used standard lease squares to examine whether the percent of extra-pair young in a nest varied with the same parameters.

Female identity and year were included in all models as random effects. Nests of plural breeding females were excluded from all analyses. We selected models based on a stepwise backward elimination of non-significant terms in order of their *P*-value. Only parameters with a *P* value of < 0.1 were retained in each final model.

RESULTS

Fledging success

Fledging success was established for 173 social groups across all years, excluding any breeding attempts by plural breeders, with 42 % of groups fledging young. Group sizes varied from 2 to 8 birds, with an average group size of 3.36 ± 1.33 SD. Of the 220 auxiliary group members, 57% were male. Mean number of male helpers per group was 0.73 ± 0.92 SD; mean number of female helpers per group was 0.54 ± 0.75 SD. Fledging success increased with group size (Table 4.3, Figure 4.1), with the presence of both male and female helpers contributing to fledging success. Broken down by groups with and without auxiliary members, 51.8% groups with auxiliaries fledged young while 26% of groups without auxiliaries fledged young.

Table 4.3. Parameter estimates of nominal logistic models of fledging success and presence or absence of extra-pair young.

Model	Parameter	Estimate ± SE	χ^2	*P*
Fledging success				
Final model	Intercept	2.39 ± 0.54	1	< **0.001**
	Group size	-0.63 ± 0.15	17.39	< **0.001**
Rejected terms	# Female auxiliaries	14.60 ± 3388111	0.17	0.67
	# Male auxiliaries	14.40 ± 20001.1	0.00	0.43
Presence or absence of extra-pair young				
Final model	Intercept	-0.75 ± 0.27	7.71	**0.005**
	# Male auxiliaries	0.51 ± 0.22	5.44	**0.02**
Rejected terms	Group size	Zeroed		
	# Female auxiliaries	0.03 ± 0.30	0.01	0.92

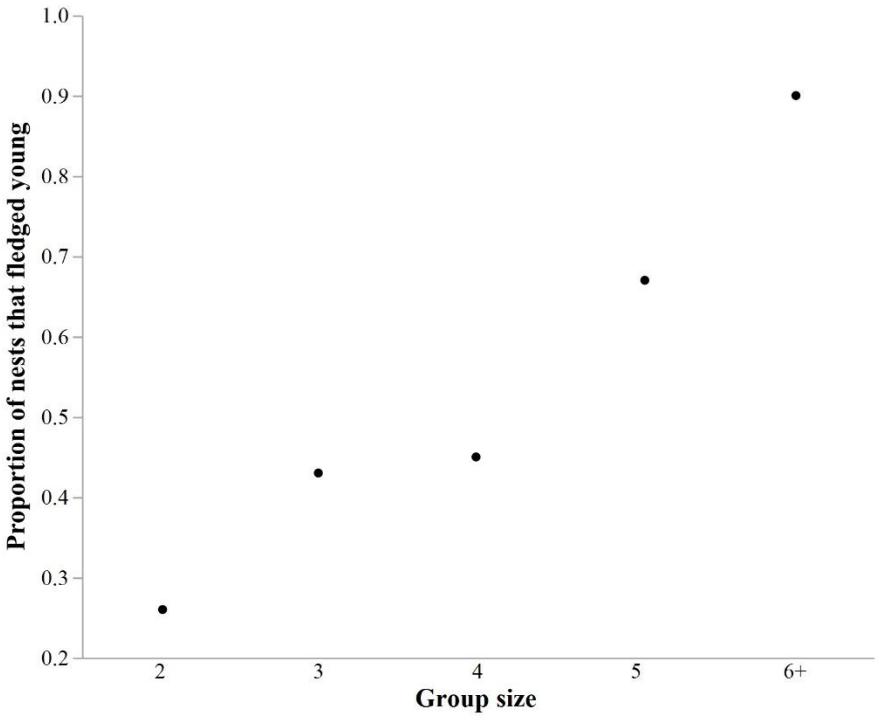

Figure 4.1. Proportion of nests of each group size (breeding birds and auxiliaries) that successfully fledged young from 2012-2014.

Egg volume and chick weight

Of the 72 clutches of eggs measured, only 59 clutches were included in subsequent analyses, including only the first nesting attempt by each female each year. Mean clutch size was 3.07 ± 0.45 SD. Because clutch size was generally stable, we also excluded one outlier clutch that contained 5 eggs, 2 of which were infertile and abnormally large. Mean egg volume was 1149.77 mm^3 ± 74.32 SD (n clutches = 59). Mean egg volume per clutch increased with Julian date of incubation initiation (clutch completion) and the number of male auxiliaries, and to a lesser degree with clutch size (Table 4.4). However, there was no effect of overall group size or of the number of auxiliary females on egg volume, which indicates there is a sex specific effect of auxiliaries on breeding female egg volume (Figure 4.2).

As above, only the first nesting attempt of each year was included in analyses of chick weight at ages 3 and 6. Mean chick weight at 3 DO was 3.23 g ± 0.72 SD (n clutches = 56); because chick weight at 3 DO deviated from normality (Shapiro-Wilk $P < 0.05$), mean chick weight at 3 DO was log transformed for subsequent analyses (Shapiro-Wilk $P > 0.05$). Mean chick weight at 6 DO was 5.91 g ± 0.75 SD (n clutches = 49), and unlike weight at 3 DO was normally distributed (Shapiro-Wilk $P > 0.05$) and was not transformed. Mean chick weight at 3 DO did not vary with any parameters (Table 4.4). Mean chick weight at 6 DO decreased slightly with the number of female helpers (Table 4.4).

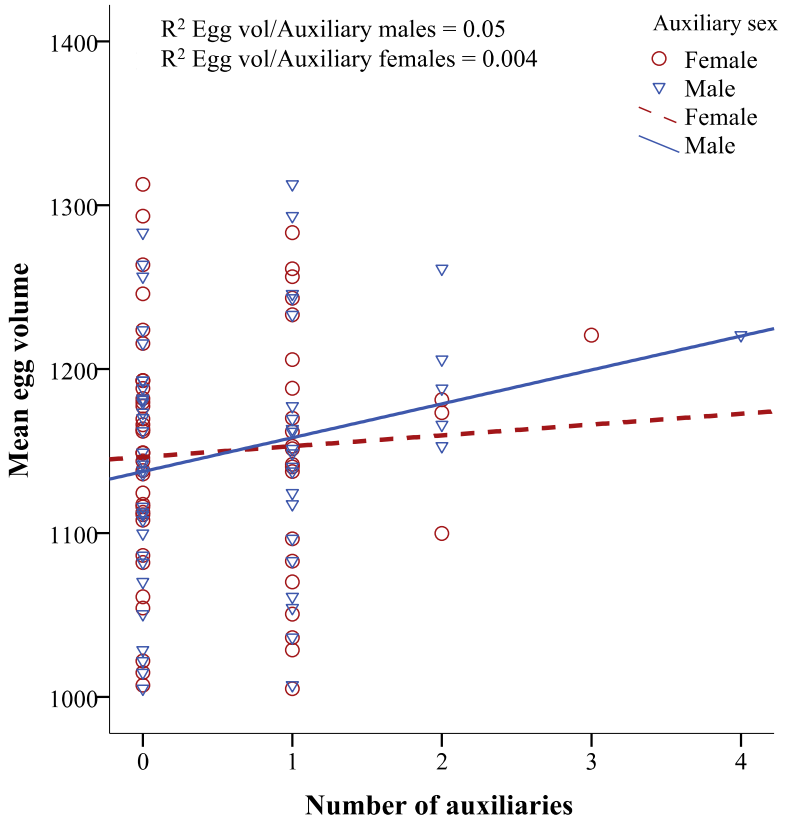

Figure 4.2. Mean egg volume within a clutch versus the number of male or female auxiliaries. Blue triangles are mean egg volume versus the number of male auxiliaries. Red circles are mean egg volume versus the number of female auxiliaries.

Table 4.4. Parameter estimates of standard least squares models of egg and nestling size and feeding rates.

Model	Parameter	Estimate ± SE	*t* ratio	*P*
Mean egg volume				
Final model	Intercept	592.29 ± 67.11	8.83	< **0.001**
	Julian date	1.50 ± 0.26	5.77	**0.01**
	# Male auxiliaries	21.06 ± 6.73	3.13	0.038
	Clutch size	33.41 ± 15.16	2.20	0.07
Rejected terms	Group size	17.39 ± 0.74	23.48	0.93
	# Female auxiliaries	8.03 ± 0.72	11.20	0.94
Log (average chick weight 3 DO)				
Final model	Intercept	3.31 ± 0.12	35.56	< **0.001**
Rejected terms	# Male auxiliaries	-0.02 ± 0.01	-1.45	0.15
	# Chicks at 3do	-0.02 ± 0.03	-0.58	0.56
	Julian date	-0.00 ± 0.00	-0.25	0.81
	# Female auxiliaries	0.002 ± 0.02	0.09	0.92
Average chick weight 6 DO				
Final model	Intercept	6.09 ± 0.22	27.71	< **0.001**
	# Female auxiliaries	-0.26 ± 0.12	-2.07	0.04
Rejected terms	Group size	-0.07 ± 0.13	-0.58	0.57
	# Male auxiliaries	-0.08 ± 0.133	-0.63	0.67
	# Chicks at 6do	-0.06 ± 0.15	-0.42	0.67
	Julian date	-0.001 ± 0.01	-0.07	0.98
Log (Total provisioning rate)				
Final model	Intercept	-0.70 ± 0.10	-7.04	< **0.001**
Rejected terms	Group size	0.01 ± 0.02	0.24	0.81
	# Male auxiliaries	-0.02 ± 0.06	-0.35	0.74
	# Female auxiliaries	-0.28 ± 0.20	-1.41	0.17
Log (Dominant provisioning feeding rate)				
Final model	Intercept	-0.79 ± 0.12	-6.48	< **0.001**
	Log dominant male rate	0.33 ± 0.09	3.78	**0.009**
Rejected terms	# Female auxiliaries	0.05 ± 0.05	1.05	0.31
	# Male auxiliaries	0.01 ± 0.05	0.29	0.77
	Group size	-0.15 ± 0.23	-0.64	0.53
Log (Dominant male provisioning rate)				
Final model	Intercept	-1.02 ± 0.11	-9.10	**0.06**
	# Male auxiliaries	-0.20 ± 0.07	-2.86	**0.009**
Rejected terms	Log dominant female rate	0.33 ± 0.2	1.63	0.12
	# Female auxiliaries	-0.04 ± 0.08	-0.48	0.64
	Group size	0.39 ± 0.34	1.63	0.11
Percent of extra-pair young				
Final model	Intercept	57.74 ± 7.24	4.761	< **0.001**
	# Male auxiliaries	-12.84 ± 5.01	-2.56	**0.02**
Rejected terms	# Female auxiliaries	-3.36 ± 6.80	-0.49	0.62

Nest provisioning

Groups observed during nest watches ranged from 2 to 8 birds, with an average group size of 3.55 ± 1.68 SD. Of the 56 auxiliaries, 59 % were male. Mean number of male auxiliaries was 0.92 ± 1.23 SD; mean number of female auxiliaries was 0.64 ± 0.90 SD. Total provisioning rate (number of feeding trips per minute) was 0.22 ± 0.11 SD. Mean dominant provisioning rate was 0.09 ± 0.08 SD; mean dominant female provisioning rate was 0.09 ± 0.05; average auxiliary provisioning rate (subordinate feeding rate averaged across all auxiliaries in the group) was 0.04 ± 0.03 SD. All provisioning rates were log transformed for statistical analyses. Overall provisioning rate at the nest was not influenced by group size, number of female or number of male auxiliaries (Table 4.4). Dominant female provisioning rate increased with an increase in dominant male provisioning rate, but did not vary with auxiliaries (Table 4.4, Figure 4.3A). Dominant male provisioning rate decreased with an increase of male auxiliaries, but did not vary with any other parameters including the number of female auxiliaries, suggesting a sex specific response to the addition of auxiliary group members (Table 4.4, Figure 4.3 B).

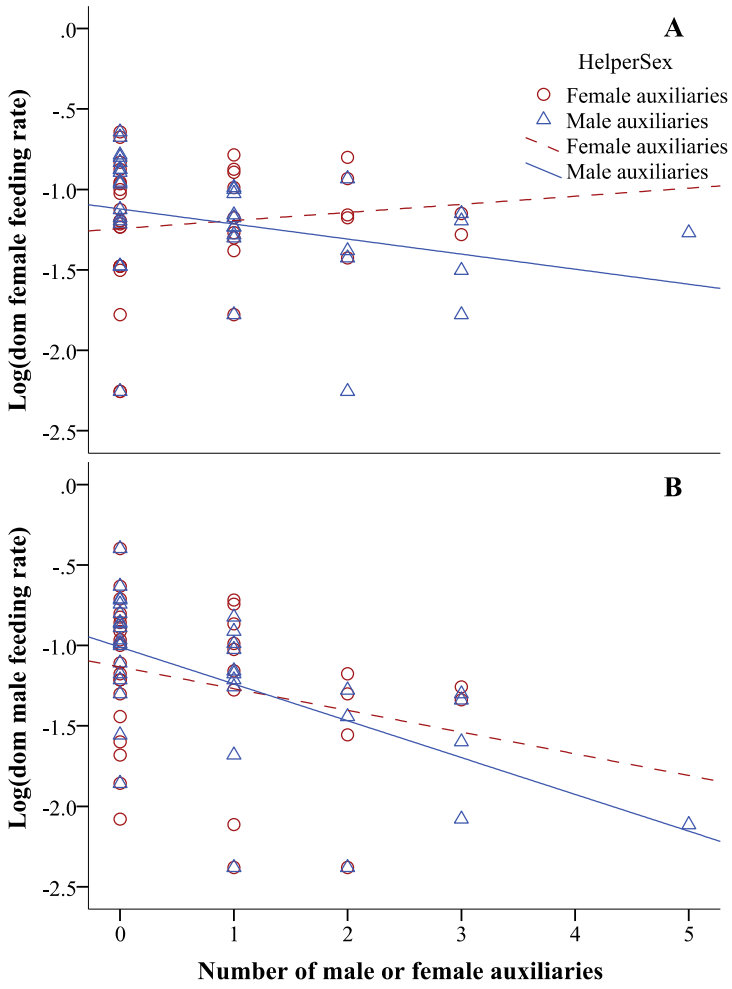

Figure 4.3. Dominant A) female and B) male feeding rate (log transformed) versus the number of male or female auxiliaries. Blue triangles are provisioning rate versus the number of male auxiliaries. Red circles are provisioning rate versus the number of female auxiliaries. Dominant female feeding rate versus number of female auxiliaries $R^2 = 0.015$, dominate female feeding rate versus number of male auxiliaries $R^2 = 0.095$; dominant male feeding rate versus number of female auxiliaries $R^2 = 0.064$, dominant male feeding rate versus number of male auxiliaries $R^2 = 0.344$.

Extra-pair paternity

Candidate fathers were accepted for 104 clutch-groups for which nestlings and group members were genotyped. Of these, 97 were retained for analysis of clutch level extra-pair paternity (presence or absence, second broods within a year and plural breeder clutches removed). Across

years, approximately 57% of groups had extra-pair young. Summary statistics for group composition and extra-pair paternity by year can be found in Table (4.5).

Table 4.5. Summary statistics for genotyped clutches across years. Second broods within each year and the clutches of plural breeds are excluded. Confidence intervals were calculated assuming a binomial distribution. Group characteristics refer only to genotyped groups.

		Clutches		Nestlings		Group characteristics	
Year	No. groups monitored	No. genotyped	No. containing extra-pair young (% ± 95% CI)	No. genotyped	Extra-pair young (% ± 95% CI)	Group size (mean ± SD)	% of aux. male
2012	36	22	10 (45.45 ± 19.89)	56	56 (56.50 ± 13.09)	4.41 ± 2.04	56.60
2013	43	34	21 (61.77 ± 14.33)	98	46 (46.95 ± 9.81)	3.12 ± 1.17	65.79
2014	70	41	26 (63.41 ± 12.69)	106	51 (48.11 ± 9.41)	3.05 ± 1.09	46.51
Total	149	97	57 (58.76 ± 9.28	260	260 (45.39 ± 6.07)	3.38 ± 1.48	55.97

Probability of having extra-pair young within a nest decreased with the presence of male auxiliaries but did not vary with the presence of female auxiliaries (Table 4.3, Figure 4.4 A). Simplified, fewer clutches of groups with male auxiliaries contain one or more extra-pair offspring than clutches of groups without male auxiliaries (47% of clutches of groups with auxiliaries and 69% of clutches without auxiliaries contain extra-pair young, Pearson's chi squared $\chi^2 = 4.32$, $P = 0.04$).

Eleven groups were excluded from the percent of extra-pair young standard least squares model because only one nestling survived to sampling age. All nests where this was the case started out with 2-3 eggs, and then lost either eggs or chicks as the season progressed. If the sampled chick was the result of an extra-pair copulation, the rate extra-pair paternity within such a clutch would be 100%, artificially inflating percent of extra-pair young. Percent of extra-pair young decreased as number of male auxiliaries increased (Table 4.4 Figure 4.4B), but does not vary with the number of female auxiliaries.

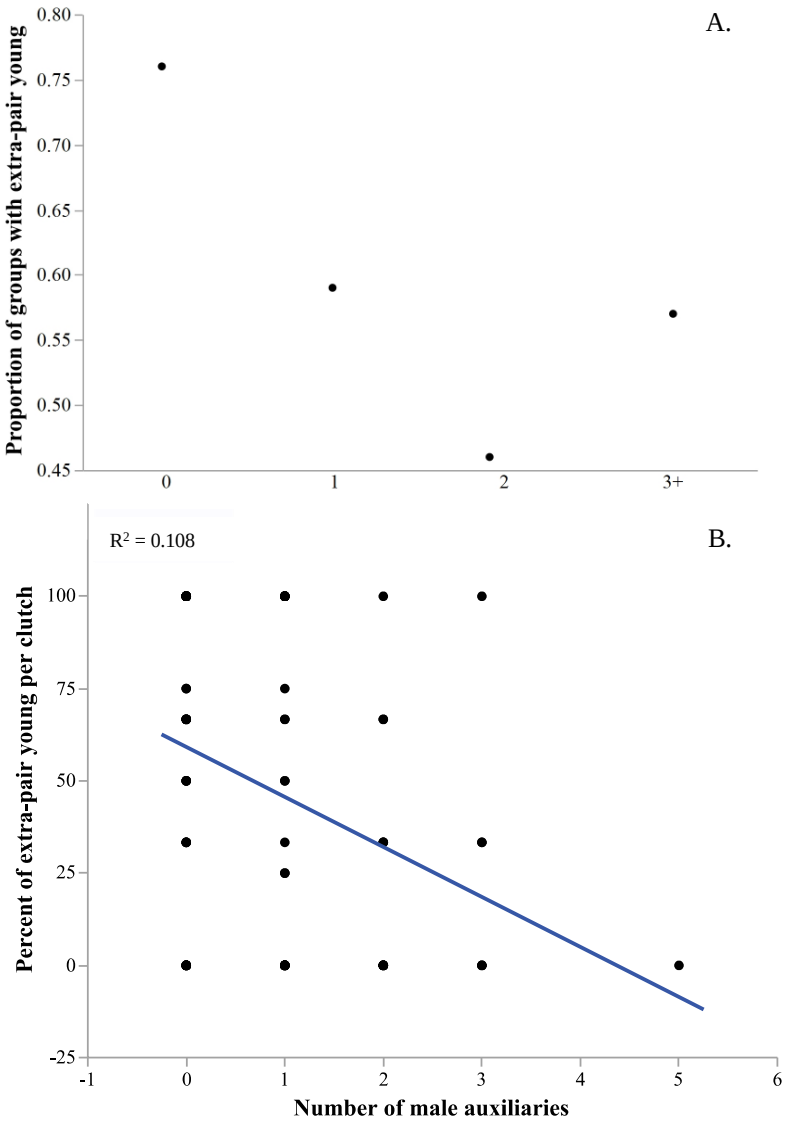

Figure 4.4. Relationship between extra-pair paternity and number of male auxiliaries. A) proportion of groups that had one or more extra-pair young as number of male auxiliaries increases and B) the percent of extra-pair young in clutch sizes of two or more versus number of auxiliary males.

DISCUSSION

How the social environment alters investment by dominant individuals has only been examined in a few cooperatively-breeding bird species (Koenig et al. 2009, Brouwer et al. 2014), and as far

as we are aware no one has examined the influence of within group social environment on extra-pair paternity rates. Using the variegated fairy-wren, a species that has high variability in both number of auxiliaries and auxiliary sex, we examined how fledging success, egg volume, nestling mass, total and dominant breeding bird provisioning rate, and extra-pair paternity change with number and sex of auxiliaries. We found that fledging success increased with both the number of male and female auxiliaries. Mean egg volume per clutch increased with Julian date of incubation initiation (time of year the clutch was laid in) and with the number of male auxiliaries, and increased slightly with clutch size, although the last parameter is not statistically significant. While average chick weight at 3 DO did not vary with any parameters, average chick weight at 6 DO increased with the number of female auxiliaries in the group. Neither total provisioning rate nor dominant female provisioning rate changed with the number of male or female auxiliaries. Female provisioning rate, however, was positively correlated with dominant male provisioning rate. Dominant male provisioning rate was best predicted by number of male auxiliaries, with a decrease in investment by the dominant male as number of male auxiliaries increased. Finally, both the rate of extra-pair paternity across clutches and the percent of extra-pair young within nests decreased as the number of male but not female auxiliaries increased.

These data suggest a strong influence of social context on fledging success, parental investment (both in provisioning as well as maternal investment in eggs) and even on extra-pair paternity rates. While male and female auxiliaries are both common in variegated fairy-wren social groups and both contribute to fledging success, there appears to be a distinct advantage to having male auxiliaries over female auxiliaries. That we see number of male auxiliaries contributing to fledging success is generally consistent with other cooperatively breeding birds. Males are most often the philopatric sex in birds (but see Berg et al. 2009), and as such are more

often “helpers.” In some systems this propensity for helping behavior translates to an increase in adult fecundity with the presence of male auxiliaries as is demonstrated in this system (Blackmore and Heinsohn 2007, Koenig et al. 2011).

In most species, the presence of helpers does not increase fecundity, but rather, results in load lightening on one or both dominant birds and or increased survival of one or both dominants (Hatchewell 1999). Female birds can alter investment in eggs (Reid 1988, Williams 1994). By reducing investment in eggs in anticipation of positive helper benefits on nestling growth, females may benefit from helpers through reduced costs of reproduction while masking positive helper effects on nestlings (Russell et al. 2007). Russell et al. found that female superb fairy-wrens reduced investment in eggs in the presence of helpers, but that the loss in egg mass was recouped as a gain in nestling mass later in the chick’s life. In contrast, acorn woodpeckers (*Melanerpes formicivorus*) show no effect of helpers on egg volume (Koenig et al. 2009); instead, females laid larger clutches in the presence of female helper. This relationship has been examined in other fairy-wren species as well, but it has not supported (Brouwer et al. unpublished data). It is striking, then, that we found the opposite pattern, with the presence of male auxiliaries resulting in increased egg volume. We suspect that this pattern may be due to higher female quality when male auxiliaries are present. Variegated fairy-wren male auxiliaries frequently feed and forage with the dominant female, therefore auxiliaries may contribute to her overall body condition during egg laying.

This increase in egg size with number of auxiliary males did not translate to an increase in nestling mass at 3 DO or at 6 DO; however, chick mass at 6 DO did increase slightly with the presence of female auxiliaries. Other species show load lightening in the presence of males, but as an additive response to provisioning in the presence of female auxiliaries (Brouwer et al.

2014). Hatchwell (1999) suggests that investment is additive in systems where starvation is frequent. While starvation was rare in the case of the red-winged fairy-wren, the authors suggested additive provisioning occurred in the presence of female auxiliaries because females were less reliable as helpers than males. Starvation may be a contributing factor in the variegated fairy-wren and was thought to occur in some cases. Starvation per se was, however, difficult to document accurately at our site, as chick death often immediately resulted in predation of the whole nest by ants, and cause and effect of the death was difficult to discern. However, because male auxiliaries exhibit higher provisioning rates than female helpers (0.04 ± 0.03 for males and 0.01 ± 0.02 for females), the increase in nestling weight at 6 DO may be the result of additive provisioning that we did not capture during the nest watches. Because nestling mass does not increase with the addition of male auxiliaries, the mechanism by which males increase fledging success is unknown. We suspect that continued provisioning and presence at the nest through stressful events (climatic or predation threat) may contribute, and we are in the process of analyzing results from simulated predation threat in groups with and without auxiliaries to begin to answer this question.

Perhaps our most notable finding is a decrease in extra-pair paternity as the number of male helpers in a social group increases. This is contrary to observations of other fairy-wren species. In both the splendid and the superb fairy-wren an increase in group size is associated with an increase in extra-pair (generally extra-group) paternity (Mulder et al. 1994, Webster et al. 2004, however see Colombelli-Négrel et al. 2009). Cuckoldry is expected to decrease parental investment by the breeding male (Burke et al. 1989, Whittingham et al. 1992, Ketterson and Nolan 1994), so females may only engage in extra-pair copulations when help by the dominant male is unnecessary, as supported by Mulder et al. (1994). Why then is there such a dramatic

reversal in the variegated fairy-wren? Because male auxiliaries influence maternal investment, parental care, and fledging success, we believe variegated fairy-wrens at this site are highly dependent on the presence of male helpers and may be near obligatory cooperative breeders. Kin selection, in coordination with delayed dispersal, is most frequently cited as the driving force behind helping exhibited in cooperative species. By remaining behind and raising potential siblings, individuals who may not have the ability or the opportunity to successfully breed can gain indirect fitness (Hamilton 1964). Because of this indirect fitness argument, cooperative species have been theorized to be highly monogamous, reducing the likelihood that helpers are unrelated to the young they raise (Boomsma 2007). Indeed, the majority of cooperative bird species are monogamous, and the transition to cooperative breeding is associated with low promiscuity (Cornwallis et al. 2010).

Fairy-wrens exhibit some of the highest rates of extra-pair paternity in birds, with 95% of broods containing at least one-extra-pair offspring in one population of superb fairy-wrens (Mulder et al. 1994). While we don't address kinship here, male auxiliaries are often sons of one or both of the breeding pair. Variegated fairy-wrens may be illustrating a transitional state from high rates of extra-pair paternity when breeding in pairs to low rates of extra-pair paternity when in cooperative groups with kin members. Alternatively, because starvation may be a factor in this population, small groups without male helpers may be likely to fail with or without the help of the dominant breeding male, so females may choose to court extra-group males to increase the genetic diversity of her offspring to increase their chances of survival. More work will need to be done to tease these possibilities apart.

Because so much diversity in ecology and group demographics exists across cooperative breeding systems, the questions of why auxiliaries are tolerated, why they delay their dispersal,

and how they “help” remain important. Further study into complex cooperatively breeding species, such as the variegated fairy-wren, explicitly focusing on social group composition may help tease apart the evolution and distribution of cooperative breeding species. Further study of the variegated fairy-wren specifically across differing social and environmental gradients may also prove fruitful to the understanding of the evolution of cooperative breeding behavior and monogamy.

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