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ABSTRACT

Cooperatively breeding species vary widely in degree of social complexity, and disentangling relationships among group members can reveal the costs and benefits of cooperation. Here, we describe the social system of the cooperatively breeding Variegated Fairywren (*Malurus lamberti*) and explore how social complexity and group dynamics may affect cooperation and conflict. We used a combination of field-based population monitoring and detailed social association observations to determine group membership annually across four breeding seasons (2014–2017) and used a ddRAD-seq genotyping method to determine genetic relationships within social groups. Social groups ranged in size from two to eight individuals and nearly half of all social groups had multiple adult individuals of both sexes. Approximately half of all groups exhibited plural breeding, in which multiple females within the same social group nested individually in the same territory. Genetic relationships were diverse among social groups due to high rates of extra-pair paternity and immigration, with most groups containing close relatives and non-relatives of each sex. Notably, although related females were often present within a social group, co-breeding females in the same social group were never closely related to each other. Given the extensive variation in relatedness among group members, cooperation in the Variegated Fairywren is likely maintained by a combination of direct and indirect fitness benefits.

Introduction

Cooperative breeding – wherein multiple individuals aid in the rearing of offspring – is widespread across taxa (Koenig and Dickinson 2016). Many cooperatively breeding animals live in family groups consisting of a single breeding pair and related non-breeding helpers (Rubenstein and Shen 2009; Koenig and Dickinson 2016). In these cases, group members receive kin selected benefits from cooperating with close relatives (Dickinson 2004; Pizzari and Gardner 2012). However, factors such as turnover of breeders and extra-pair paternity (EPP) can complicate patterns of genetic relatedness among individuals within a group, potentially leading to reduced inclusive fitness benefits (Hamilton 1963, 1964; Bourke 2014). Nearly half of cooperatively breeding bird species live in social groups containing some combination of unrelated and related individuals (Riehl 2013). Yet the vast majority of studies of cooperative breeding have focused on species living in family groups (Hatchwell and Komdeur 2000; Shen *et al.*

2017), and studies of species that live in more genetically complex groups are far less common (Painter *et al.* 2000; Clutton-Brock 2009; Riehl 2013), thus constraining our understanding of the evolution of cooperation.

When genetic relationships among cooperative group members are mixed, kin-selected benefits are diminished for some group members, and other benefits or constraints are likely necessary to explain cooperation among non-relatives (Painter *et al.* 2000; Dickinson 2004; Clutton-Brock 2009; Kingma *et al.* 2011; Carter and Wilkinson 2015). For instance, group members may benefit from the opportunity to fill vacated breeding roles (Cockburn *et al.* 2008; Shen *et al.* 2017), which is more likely to occur in groups containing multiple breeding pairs. Cooperation can also allow species to buffer against detrimental effects of inhabiting unpredictable environments (Rubenstein and Lovette 2007) and enhance defence against predators and brood parasites (Kokko *et al.* 2001; Feeney *et al.* 2013). Cooperatively-breeding species that exhibit both

unrelated and related individuals cooperating are particularly interesting because multiple types of benefits of cooperation may operate simultaneously (Dickinson 2004; Clutton-Brock 2009; Rubenstein *et al.* 2016).

The Australasian Fairywrens (*Malurus* spp.) have served as model systems for studies of cooperative breeding and complex social behaviour for several decades (Buchanan and Cockburn 2013; Cockburn *et al.* 2013; Joseph *et al.* 2013). Many Fairywren species live in family groups, with a breeding pair and several helping (often male) auxiliaries (Rowley and Russell 1997). However, genetic relatedness in these species can be complicated by high EPP rates (Webster *et al.* 2004; Varian-Ramos and Webster 2012; Cockburn *et al.* 2013; Brouwer *et al.* 2017) and rapid replacement of breeders when a vacancy opens (Varian-Ramos and Webster 2012). In some Malurids, female auxiliaries also serve as non-breeding helpers (Russell and Rowley 2000) and are often daughters that delay dispersal and help in their natal group. Independent reproduction by multiple pairs within social groups (i.e. plural breeding) is uncommon in Fairywrens (but see Rowley *et al.* 1989; Buchanan and Cockburn 2013) but appears to be more likely when immigrant females join established groups (Brouwer *et al.* 2011; Johnson and Pruett-Jones 2018). Thus, high EPP rates and immigration can lead to complex patterns of relatedness within social groups, likely affecting the relative costs and benefits of group membership.

Here we examine the group composition and social dynamics in the Variegated Fairywren (*Malurus lamberti*) of eastern Australia, a member of the 'chestnut-shouldered clade' of Fairywrens, recently split from its sister species, the Purple-backed Fairywren (Mclean *et al.* 2017a, 2017b). Natural history studies for this species have shown that social group size can vary substantially, and female auxiliaries of unknown reproductive status have been reported (Rowley and Russell 1997). Despite being a rather conspicuous and common species, little is known about its social system, particularly genetic relationships of group members and breeding behaviour. We intensively monitored a population of Variegated Fairywrens near Brisbane, Queensland, to study the social dynamics and genetic relationships of this cooperatively breeding species. We used detailed field observations to describe overall associative behaviour and territoriality, determined group size and composition and designated social statuses within groups. In one study year, we conducted social network observations to ground truth group assignments from casual surveys. Additionally, we used a unique panel of single nucleotide polymorphisms (SNPs) to determine the relatedness of adults within social groups and assess extra-pair paternity. Together, these approaches allowed us to show that this species

exists in especially complex social groups, often containing multiple breeding pairs and helpers, with relationships among group members varying from completely unrelated to parent–offspring relationships.

Methods

Study population and general field methods

We studied a population of Variegated Fairywrens (*M. lamberti*) around Lake Samsonvale (27°160 S, 152° 410 E), 30 km northwest of Brisbane, Queensland, Australia (see Figure S1 for map of study site). We collected data on colour-banded birds throughout the breeding season each year, typically August – January, from 2014 to 2017. The predominant habitats at our study site include subtropical grasslands, eucalypt plantations, and dry eucalypt forests with secondary growth. Variegated Fairywrens at our study site largely occupied areas of secondary growth but were also present to a lesser extent in other habitat types, such as open grassland and lake margins. We captured birds using targeted mist-netting, occasionally using playback of distress calls as a lure. We banded each adult with a unique combination of three plastic colour bands and an Australian Bird and Bat Banding Scheme (ABBBS) aluminium band for individual identification. We collected a jugular blood sample of 50–80 µl from all individuals for subsequent genetic analyses (Baldassarre and Webster 2013), as well as standard morphometric measurements including mass, tarsus and tail length, wing chord and bill measurements. When possible, we determined the age and sex of each captured individual using plumage characteristics (i.e. nuptial plumage, Johnson and Pruett-Jones 2018), ossification of the skull (Lindsay *et al.* 2009), and physical indicators of reproductive status (i.e. brood patch or cloacal protuberance). Throughout each breeding season we systematically monitored the population by assessing group membership, affiliative behaviours and breeding activity. The geography of the field site (i.e. bordered on multiple sides by water) also afforded close monitoring of dispersal and movement of individuals born into the population, and immigrants entering the population.

Social group composition

We employed two methods to determine social group composition. First, in every year of the study, social group membership and territories were determined through routine monitoring of individuals across the study site. Initial social group assignments were made in the first few weeks of each field season by identifying

social groups as any aggregation of two or more individuals that were present in the same area on more than three occasions, and that were observed engaging in associative behaviour (e.g. allopreening and foraging in close proximity) with each other or in coordinated defensive behaviours (i.e. territoriality) against conspecifics. After initial group assignments, each social group on the field site was observed approximately once every 3 days or more frequently throughout the remainder of the season. These repeated observations allowed us to confirm our initial designations, assess any changes in social group composition, and determine female breeding status. Although most groups were stable within a breeding season, some did undergo changes in size and/or composition. In these cases, to avoid pseudoreplication, we considered the group's most complex arrangement of adult birds within each season for subsequent analyses, defined as being the largest in size, having multiple females (breeding or non-breeding), and/or having multiple breeding females. In most cases, an individual's most complex group was its first social group of the season.

Second, we conducted additional structured observations of associations among individuals during the 2016 field season only, and analysed these data using social network analysis to corroborate our routine monitoring group assignments described above. This method of structured observations and social network analysis has previously been applied to assess Fairywren social interactions and define social group structure (Welklin *et al.* 2023). Our structured observations consisted of 25-min focal follows of social groups, during which we collected data on which birds were associating every 5 min, resulting in six sampling points per observation. In this method, birds were considered associating if they were within 30 m of each other and moving and vocalising in a coordinated manner leading up to the sampling point, as a greater distance or lack of coordination suggested individuals were unaware of each other and not interacting. Most social groups were followed for at least three 25-min observations (mean = 5.82 observations/group). We constructed a social network for the 2016 season using the gambit of the group method, considering any individual associating in a sampling point to be associating in the network (Welklin *et al.* 2023). We built the network using the simple ratio index (SRI) in the R package 'asnipe' (Farine and O'Hara 2013), then removed individuals that were seen fewer than seven times, meaning an individual had to be seen in at least two observations to be included in the network. Analysis of the relationship between times seen and degree (number of associates) revealed that removing individuals seen fewer than seven times resulted in

a non-significant relationship ($p > 0.05$) between times seen and degree. This step ensured that the structure of the social network structure was not biased by individuals with little data.

We identified social groups in the social network using a dendrogram method (see Welklin *et al.* 2023). To summarise, we created a dendrogram using the UPGMA method, then searched for the bifurcation point in the dendrogram that was associated with the highest average silhouette width when the dendrogram was cut at that point. Silhouette width is a clustering quality score that compares the distances between nodes (individuals) within a cluster (social group) to the distance to the next-closest cluster. A score close to 1 indicates a network with distinct clusters and a score close to 0 indicates a very uniform network. Individuals alone in solo 'groups' were removed from the network as we never observed floaters in our population, and it was more likely that other group members associated with these individuals were not observed often enough to be included in the network. We compared the structure of these network-defined groups to those from data-stream permuted networks to test whether the observed groups were more structured than expected by random chance (Welklin *et al.* 2023). We compared the social group membership of the network-defined social groups to those identified by routine sampling by calculating the percentage of within-group dyads that were in the same groups across the two different methods.

We found the vast majority of nests ($N = 15$ nests fledged before being found; 1.5% of known nest attempts) and intensively monitored nesting attempts for all social and breeding groups throughout each breeding season. Females within each group were designated as 'breeding' if they were observed actively engaging in nest-building, incubation or brooding, or if they were captured with an active (i.e. defeathered and vascularised) brood patch. In Fairywrens, only breeding females build nests and incubate eggs (Schodde 1982; Rowley and Russell 1997). Males that attended to the female closely throughout nest building and early nestling rearing were designated as breeding social mate(s) of the breeding female. We designated co-breeding females as primary, secondary, or tertiary based on their tenure and seniority within a social group. The first female occupying a breeding position was designated as primary and immigrant females that joined established groups as breeding females were designated as secondary or tertiary. When this information was not available, the first nesting female was designated as the primary and any subsequent nesting females were designated as secondary and tertiary. Auxiliary individuals within a group were those without their own nest (i.e.

females that never built a nest and males that were not associated with a nest-building female). When nestlings reached 6 days of age, they were banded with an aluminium ABBBS band, and we took a small tarsal blood sample for genetic analysis of parentage.

SNP genotyping and pairwise relatedness

We used a diverse set of single nucleotide polymorphisms (SNPs) to address questions about genetic relatedness among individuals in social groups. The SNP panel was derived using a double-digest restriction-site associated DNA sequencing approach (ddRAD-seq) described in Thrasher *et al.* (2018). A total of 858 individuals were sequenced across five sequencing runs using an Illumina HiSeq 2500 with single-end reads. Following sequencing, we used a *de novo* assembly for subsequent SNP calling. After filtering for missing data, depth of coverage, and minimum allele frequency, the SNP panel consisted of 358 unique markers (see Thrasher *et al.* 2018 for filtering metrics).

We used the package, 'RELATED' (Pew *et al.* 2015), in R version 3.5.3 (R Core Team 2019) to estimate pairwise relatedness (*r*) for all adults within each social group. This package accounts for genotyping errors and missing data and can estimate relatedness using any of seven different estimators (four non-likelihood-based and three likelihood-based). Using the *compareestimators* function, we generated simulated data from observed allele frequencies and assessed the performance of different non-likelihood estimators on the simulated data. We generated 200 simulated pairs of individuals for each degree of relatedness (i.e. half-sib, full-sib, parent–offspring and unrelated) and determined that the Wang (2002) estimator provided simulated estimates that best matched the observed data (see also Thrasher *et al.* 2018). Using the Wang (2002) estimator, we again generated 200 pairs of individuals for each degree of relatedness using the *familyism* function. The distributions generated from this function provided the bounds for assigning relationships when the values deviated from each predicted degree of relatedness. We then calculated pairwise relatedness between all individuals with the Wang (2002) estimator using the *coancestry* function (Wang 2011). These estimates were subsequently used to determine the genetic relationships between breeders and auxiliaries and between co-breeding individuals.

Paternity analysis

Paternity assignments and estimates of extra-pair paternity (EPP) were assessed for all years of this study (2014–17). CERVUS version 3.0.7 (Kalinowski *et al.* 2007) was used to assign paternity for all nestlings sampled using a two-step, likelihood-based approach to assign parentage. First, CERVUS compares each offspring's genotype to that of a candidate parent and a random individual in the population to calculate the likelihood ratio. This relationship is presented as an LOD (logarithmic of the odds) score, which is simply the natural logarithm of the calculated likelihood ratio. Positive LOD scores indicate that a candidate parent is much more likely to be the true parent, whereas negative LOD scores indicate that the candidate parent is highly unlikely to be a true parent. Second, CERVUS conducts a simulation of parentage analysis based on population allele frequencies and the proportion of potential parents included in the analysis. The simulation accounts for the possibility of unsampled parents, missing data and genotyping errors. Considering these parameters, the simulation calculates critical LOD scores by comparing the LOD distributions of the most likely parent and all other candidate parents. The critical LOD score is used to determine the confidence (95% or 80%) of each parentage assignment.

Paternity was analysed separately for each breeding season and included known mothers for each brood and all candidate males from the entire population. For each breeding season, we used genotypes of adults to simulate paternity assignments for 10,000 offsprings to determine critical LOD scores for exclusion of non-fathers. Simulations across years included the following parameters: 95% of candidate males sampled, an estimated error rate of 0.01 for mistyped loci and likelihood scores, and a varying number of candidate males depending on year (2014: 150; 2015: 134; 2016: 135; 2017: 146) The average proportion of loci typed across all individuals for all years was 0.92. We used the trio LOD score and the father–offspring LOD score from CERVUS to make assignments. The trio LOD score was calculated by comparing the genotypes of the candidate male and offspring, relative to that of the known mother. The father/offspring LOD score only accounts for the relationship between the candidate male and the offspring, independent of the known mother. CERVUS ranked candidate males by LOD scores in each category, and the highest-ranking males were assigned as fathers. These rankings should be in agreement, but ambiguous assignments (different top ranking males assigned in each category) may occur when multiple candidate male genotypes closely match an offspring's genotype.

We assessed each CERVUS assignment to determine whether it was plausible and accepted the CERVUS assignment if the highest-ranking male was in agreement for both the trio LOD and the father-offspring

LOD, and if the number of mismatches between the assigned male and the offspring was less than the observed maximum number of mismatches between known mothers and their offspring (max. = 8, 2.2% of 358 loci, mean = 2 mismatches). We accepted the social father as the genetic sire if they met these respective criteria. If the social father mismatched the offspring at higher numbers, or had negative LOD scores, the offspring was considered sired by an extrapair father. We accepted assignments of extrapair fathers using the same criteria outlined for acceptance of social fathers. When offspring could not be assigned to their social father or any sampled extrapair sires, we considered them to be sired by an unsampled extrapair sire.

Statistical analyses

We used R version 3.5.3 (R Core Team 2019) to compile social data and calculate summary statistics. All plotting was done in base R and ggplot2 (Wickham et al. 2016).

Ethical note

All field methods were approved by the Cornell Institutional Animal Care and Use Committee (IACUC 2009–0105), Tulane University IACUC (2019–1715), and the James Cook University Animal Ethics Committee (A2100). The present study was permitted under Queensland Government Department of Environment and Heritage Protection Scientific Purposes Permit (WISP15212314). Our banding and blood sampling methods have been used previously in a closely related species with no observable negative effect (Webster et al. 2008).

Results

Group size and composition

From 2014 to 2017, we captured and sampled 858 individuals (319 adults and 539 nestlings). The number of social groups monitored ranged from 54 to 57 groups across all 4 years of the study (Table 1). Territories remained largely stable between years, but social groups changed due to demographic processes, so independent social groups were identified each year. In total, we identified and monitored 222 unique social groups during the study (Table 1, Figure 1).

In the 2016 field season, we compared the group membership of 170 individuals in 50 social groups and found that nearly all (95%) of dyadic relationships that occurred within routine monitoring groups were also present in the network-defined groups (Figure 2). The few mismatches between these methods can be

Table 1. Number of Variegated Fairywren social groups, average group size, and composition by year.

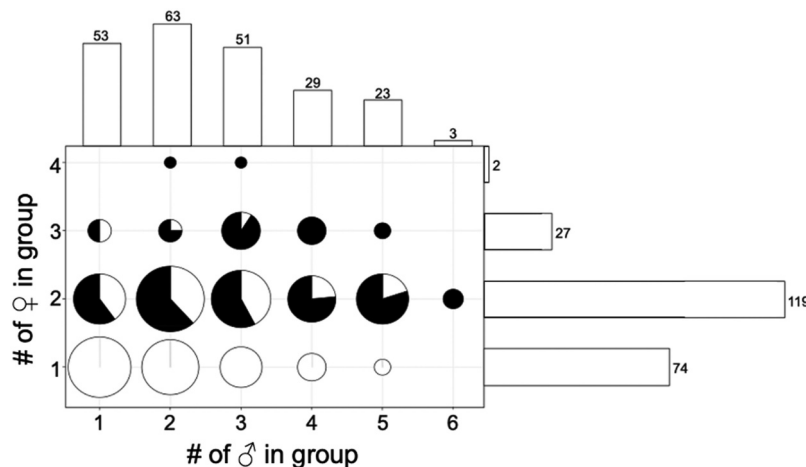| Year	No. of groups	Group size (mean ± SD)	No. of males (mean ± SD)	No. of females (mean ± SD)
2014	55	4.58 ± 1.55	2.84 ± 1.34	1.75 ± 0.62
2015	54	4.52 ± 1.50	2.70 ± 1.24	1.81 ± 0.52
2016	57	4.23 ± 1.76	2.44 ± 1.36	1.79 ± 0.73
2017	56	4.38 ± 1.74	2.50 ± 1.36	1.88 ± 0.81
Mean	55.5	4.42 ± 1.64	2.62 ± 1.33	1.81 ± 0.67

Figure 1. Composition of Variegated Fairywren social groups from 2014–2017. The size of each circle indicates how common that composition was relative to the total number of groups (N = 222). Histograms along both axes show the number of groups with each number of individuals by sex. The black portion within circles indicates the proportion of groups that had multiple breeding females (as opposed to auxiliaries), and white indicates the proportion of groups with a single breeding female.

explained by a small number of groups: in one instance, the network analysis split a pair that was together in the field-defined groups; in another instance, a single bird was placed in a different group in the network analysis, and there were two instances where the network and database differed on whether to split a large group or to keep it together. Permutation analyses revealed that the network-defined groups were more structured than expected by chance ($p < 0.01$, Figure S2). Together

these results validate the social group assignments determined through our routine sampling method.

Social group size ranged from two to eight adults (Table 1) and the adult sex ratio was male-biased (1.44:1). The number of males in a social group was more variable than was the number of females (Table 1, Figure 1), ranging from one to six males and one to four females per group. Of all social groups, 13% were socially monogamous pairs ($n = 29$), 40% were cooperative
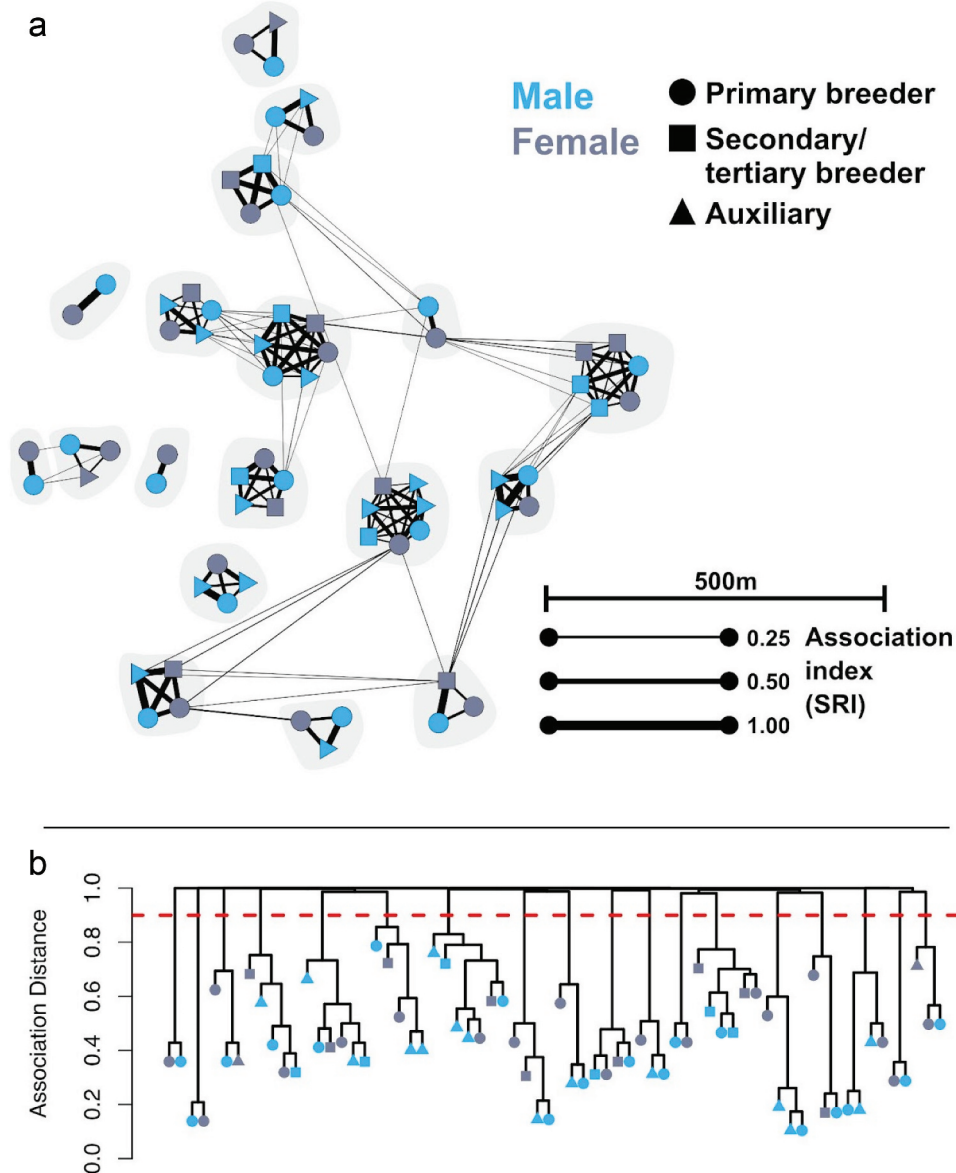

Figure 2. Variegated Fairywren social relationships and group structure during the breeding season. a) A representative subset of the social network from the 2016 breeding season. Each node represents an individual bird and lines connecting nodes are sized relative to how often those two individuals were seen together using the Simple Ratio Index (SRI). Thicker lines indicate individuals seen associating more often than thinner lines. Sex is represented by colour, breeding status is represented by node shape, and social group membership is represented by shading behind nodes. Social groups are plotted geographically to most common observation locations for each group. b) Dendrogram used to identify social group membership for the subset of individuals in the network above. Each node represents an individual bird as above and individuals connected by lines that do not cross the horizontal red line are considered in the same social group. Association distance (y-axis) is the inverse of the association index (1-SRI).

groups, with one breeding female ($n = 88$), and 47% were cooperative groups with multiple co-breeding females ($n = 105$). Groups with multiple females were common (67%; $n = 148$; Figure 1), and over two-thirds of those exhibited plural breeding (i.e. multiple co-breeding females within a single social group). Of these plural breeding groups ($n = 105$), 90% had two breeding females and 10% had three breeding females.

Origins and statuses of known individuals

We followed 115 nestlings (74 males; 41 females) to adulthood and identified 60 yearling immigrants (19 males; 41 females) during our study (Table S1). In their first year, males were typically philopatric (89% of males), whereas most females dispersed from their natal territory and off our study site as yearlings (59%; Table S1). Of the yearling dispersing females remaining on our study site, 3 (12.5%) joined neighbouring groups as auxiliaries, 6 (25%) filled a breeding vacancy as the sole breeding female in the group, and 15 (62.5%) joined a group as a secondary or tertiary co-breeding female. Females that did not disperse as yearlings did so the following year, except one female that remained on her natal territory and became the primary breeding female in her fourth year after the disappearance of her mother. This was the only case of a female inheriting a breeding position on her natal territory during our study.

Sexes differed in their likelihood of adopting a breeding vs. auxiliary role. Of those hatched on territories within our study site ('local' females and males), most local females (61%, $N = 25$ of 41 females) became breeders during either their first (37%, $N = 15$) or second (24%, $N = 10$) breeding seasons, and the remaining 39% ($N = 16$) spent their first season as an auxiliary and then disappeared (presumably dispersed outside of study area or died); none of these females remained as an auxiliary for more than one season. In contrast, a large proportion of the local males hatched on the study site remained as non-breeding auxiliaries (39%; $N = 29$ of 74 males) throughout the study, 20% ($N = 15$) adopted a breeding role in their first and 24% ($N = 18$) became breeders in their second breeding season. Only 16% ($N = 12$) of auxiliary males disappeared after spending their first season as a non-breeder. Immigrants were more likely to adopt a breeding role in their first breeding season on our study site in both males (53%; $N = 10$ of 19) and females (66%; $N = 27$ of 41).

Relatedness within social groups

Average pairwise relatedness of adults within social groups was 0.11 (SD ± 0.21), suggesting a mix of closely related and unrelated individuals. Within a social group, pairwise relatedness estimates were generally much higher between males than between females (males: mean ± SD = 0.18 ± 0.20, $n = 495$; females: 0.06 ± 0.20, $n = 147$), suggesting that many females were immigrants to the group and unrelated to one another, as shown in observational data (Table S1).

We further investigated relationships among group members by assessing patterns of pairwise relatedness between different categories of group members (Figure 3). Co-breeding females were mostly unrelated to each other (mean ± SD = −0.01 ± 0.07; Figure 3(a)), as would be expected if breeding females were mostly immigrants from other social groups, which is supported by our behavioural observations (see above). Pairwise comparisons between breeding and non-breeding females revealed a bimodal distribution (Figure 3(b)), one mode of highly related individuals (r-value ~0.50), as expected of mother-daughter or sister pairs, and the other corresponding to completely unrelated individuals (r-value ~0.0). The distribution of relatedness of males within social groups was more continuous due to individuals of varying degrees of relatedness between the expected values for unrelated individuals and full sibs ($r = 0.0$ and 0.5, respectively; Figure 3(d)). Pairwise relatedness between breeding females and all males within a social group (Figure 3(c)) was similar to that of breeding females and non-breeding females (Figure 3(b)), but with fewer relatives. The distribution of pairwise relatedness between non-breeding females and all males also exhibited a bimodal distribution of unrelated individuals and highly related individuals, but the majority were predominantly unrelated (Figure 3(e)).

Extrapair paternity

We were able to assign 357 offsprings (98.6%) to a genetic sire above the 95% confidence level based on the trio LOD score. The remaining five offspring (1.4%) did not match well with any sampled male and so were determined to be sired by unsampled extrapair males likely from outside of the main study area. EPP levels were consistently high for each year of the study. For all years combined, 80% of the sampled broods ($N = 130$) had at least one extrapair offspring, and 60% of all sampled offspring ($N = 214$) were sired by an extrapair male (Table 2).

Discussion

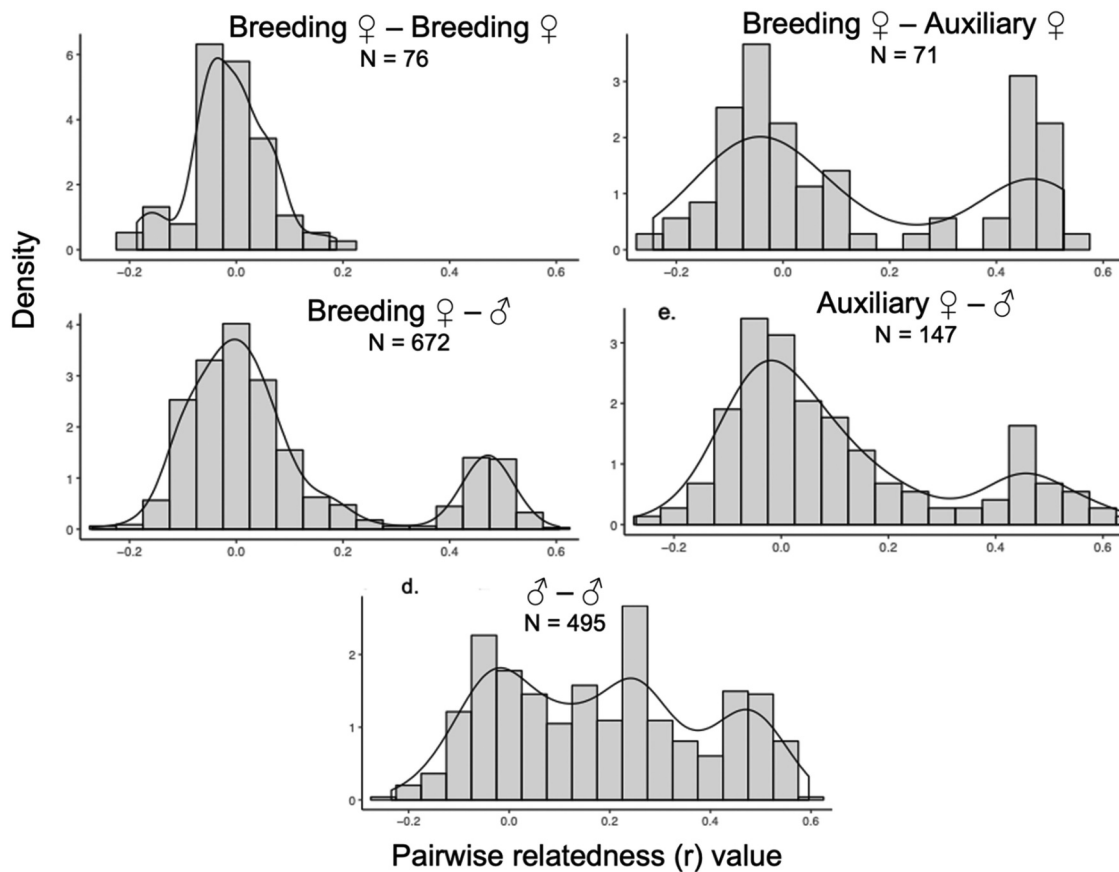Our study reaffirms that Variegated Fairywren is a cooperative breeder (Buchanan and Cockburn 2013;

Figure 3. Distributions of pairwise genetic relationships of Variegated Fairywren social group members based on sex and breeding status of females. Comparisons include those between a) breeding females, b) breeding females and non-breeding females, c) breeding females and all males, d) non-breeding females and non-breeding males, and e) all males. Note varying y-axes across panels.

Table 2. Number of Variegated Fairywren nests, percentage of nests with at least one extra-pair young (EPY), brood size, number of nestlings, and percentage of extra-pair young across nestlings by year.

Year	No. of nests	% with EPY	Brood size (mean ± SD)	No. of nestlings	% EPY
2014	35	80%	2.74 ± 0.70	96	67%
2015	28	82%	2.93 ± 0.47	80	58%
2016	42	79%	2.71 ± 0.60	113	68%
2017	25	80%	2.76 ± 0.60	68	46%
Mean	32.5	80%	2.79 ± 0.59	85	60%

Mclean *et al.* 2017a, 2017b) and shows that the breeding system involves highly complex patterns of relatedness and reproduction within social groups. Although some groups were socially monogamous pairs that reared and fledged offspring in the absence of auxiliary helpers, most groups (nearly 87%) were cooperative groups with auxiliary adults. Cooperative groups were extremely variable in size and composition, ranging widely in number of males and females occupying breeding or auxiliary roles (Figure 1). Although most groups were male-biased, well over half of all social groups contained multiple females, and of those a large majority (71%) were groups that contained two or more breeding females. Multi-female groups in our population formed

as the result of recruitment of daughters on their natal territory, the arrival of immigrant females to a group with an established breeding female, or combinations of the two. Plural breeding groups always manifested through immigrant females joining groups with established breeding females. This pattern was confirmed by our genetic analysis, which showed that co-breeding females were never close relatives.

Social structure and extra pair paternity

Large group sizes in the Variegated Fairywren could be incentivised through increased capacity to combat predators or brood parasites and breed despite variable

environmental conditions (Hatchwell and Komdeur 2000; Rubenstein and Lovette 2007; Feeney *et al.* 2013; Shen *et al.* 2017). However, Red-backed and Superb Fairywrens breeding at the same site form substantially smaller breeding groups on average than Variegateds despite all being subjected to the same nest predator and brood parasite species and abiotic conditions. Differences in group size among these cooperatively breeding congeners are likely due to intraspecific variation in response to the same environmental conditions, as was shown recently in Superb and Purple-backed Fairywrens along an environmental gradient (Johnson *et al.* 2023) Reforestation efforts at our study site have seemingly improved Variegated habitat at the expense of open savanna habitat for Red-backed and Superb Fairywrens (Webster *et al.*, personal observation), which could also explain larger group sizes in the former besides potential fixed intraspecific differences in sociality.

Groups identified via social network analysis in 2016 matched the groupings assigned through routine monitoring to almost exactly (95%), thus lending confidence in our group assignments across study years. We often observed interactions between individuals assigned to different groups (Figure 2) and suspected that most connections between social groups in our study were the product of individuals searching for breeding vacancies and engaging in extra-pair courtship (Welklin *et al.* 2023). Fairywrens exhibit unusually high levels of extra-pair paternity (EPP) with both males and females embarking on off-territory forays in search of extrapair mating opportunities (Rowley and Russell 1990; Double and Cockburn 2000; Potticary *et al.* 2016; Leitão *et al.* 2019; Boersma *et al.* 2022). In our study, 80% of nests contained extra-pair young, with 60% of nestlings in the population sired by males not mated to the breeding female (Table 2). High EPP in this system likely results both from extra-group mating and other breeding or auxiliary males in the group siring young. Forthcoming studies will explore whether EPP varies by social role and degree of relatedness among group members.

Relatedness within social groups

One of the most striking patterns revealed by our genetic analysis was that plurally breeding females within a group were always unrelated to one another. While plural breeding has been noted in other Fairywrens, co-breeding females in those species were typically close relatives (i.e. mothers and daughters; Rowley *et al.* 1989; Russell and Rowley 2000). In other species, such as the Galapagos Mockingbird (*Mimus parvulus*; Curry 1988) and Mexican Jay (*Aphelocoma wollweberi*; Barkan *et al.* 1986; Li and

Brown 2000), plural breeding by close relatives is common and is usually the result of limited breeding opportunities outside of the social group. Secondary breeding females in these species often initiate their own nests, but generally at a lower level than primary breeders. In our study plural breeders were always non-relatives, which is uncommon in birds (Riehl 2013), and the first published evidence in Fairywrens. Secondary and tertiary breeding females often had their own mate, but in some cases shared a mate with the primary or another co-breeding female (Figure 2(a)). Co-breeding females jointly defend territories with other members of the social group, so could benefit from enhanced capacity to defend limited resources and deter predators and brood parasites (Riehl and Jara 2009; Feeney *et al.* 2013; Shen *et al.* 2017). Determining the fitness consequences for plural breeding will be informative to our understanding of mating systems and the evolution of cooperation.

Relatedness patterns of males within social groups were a mixture of nonrelatives ($r = 0$), moderate relatives ($r = 0.25$) and close relatives ($r = 0.5$). Varying levels of relatedness among males in the same social group are likely the product of high EPP rates, and in rarer cases, immigrant males joining groups. The extent to which offspring are related to breeding pairs can vary with the degree to which they help with provisioning and whether they attempt their own reproduction within the group (Williams 2004; Riehl and Jara 2009; Raihani *et al.* 2010; Groenewoud *et al.* 2018). Consistent with other Fairywren species, greater female dispersal led to a strong male sex bias among auxiliaries in our study (Russell and Rowley 2000; Webster *et al.* 2004; Potticary *et al.* 2016; Johnson and Pruett-Jones 2018; Leitão *et al.* 2019).

Given the complexity of relatedness within social groups, it is likely that cooperation among adults is maintained via both indirect and direct fitness benefits (West *et al.* 2007). For both sexes, some individuals remain on their natal territories as non-breeding helpers, likely deriving some kin-selected benefits (West *et al.* 2007; Kingma *et al.* 2010; Bourke 2014), though direct benefits are also possible. This strategy was common in males, but much fewer females remained as non-breeding helpers beyond their first year. Female helpers likely benefit from the capacity to fill vacated breeding positions or mate with immigrant males. Immigrants of both sexes often joined established social groups as unrelated auxiliaries, with most eventually adopting a breeding role in the group they joined, suggesting that non-kin auxiliaries are immigrants that join the group to queue for breeding opportunities. Unrelated male auxiliaries may also benefit from

sneaking copulations with females (Riehl 2013). Future work in this system can resolve the selective pressures underlying complex sociality.

Conclusions

Our findings indicate that Variegated Fairywrens exhibit a complex social system characterised by a dynamic combination of breeding and non-breeding individuals of varying relatedness. This species is unique among *Malurus* Fairywrens in that unrelated co-breeding females are often present in the same social group. The complexity of this social system offers an ideal opportunity to answer questions about cooperation and conflict in social groups. Mixed relatedness due to high EPP rates and immigrants joining groups suggests that the benefits of cooperation are likely a mix of indirect (kin selection) and direct benefits. Unrelated co-breeding females cooperate in territory defence and thus might derive benefits from enhanced protection from conspecifics, predators, and brood parasites. Plural breeding may also incentivise cooperation in males as opportunities for breeding are more likely as the number of breeding females increases. Continued work in this system will explore which factors contribute to cooperation among individuals of varying relatedness and the relative costs and benefits of variable social roles. Resolving whether group size varies with environmental changes and prevalence of brood parasites will provide important insights into the evolution of cooperative breeding in this system. Variations in group size in our study population coupled with cooperation among group members of varying relatedness provide an ideal opportunity to test competing theories of the evolution of cooperation.

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Disclosure statement

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